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VOLUME 101

ACTA PHYSIOLOGICA SCANDINAVICA

Vol. 101 INDEX

Fasc. 1 (September 1977)

Fasc. 2. (October 1977)

Short Communication.

Fasc. 3. (November 1977)

Short Communications

Fasc. 4. (December 1977)

FASC. 2. (October 1977)

Short Communication

Acta physiol. scand. 1977 101. 1–14

From the Institute of Medical Physiology B, University of Copenhagen, Denmark

Capillary Permeability in Skeletal Muscle

By

WILLIAM P. PAASKE

Received 17 January 1977

Abstract

PAASKE, W. P. *Capillary permeability in skeletal muscle.* Acta physiol. scand. 1977. *101.* 1–14.

Capillary permeability in skeletal muscle was investigated by applying the single injection, external registration method to the autoperfused cat gastrocnemius preparation. ^{51}Cr-EDTA (MW 341.2) and ^{57}Co-B12 (MW 1353.5) served as simultaneously injected indicators. At a plasma flow of 7 ml/100 g min and an assumed capillary surface area, S, of 70 cm^2/g the permeability coefficients P_d(^{51}Cr-EDTA) and P_d(^{57}Co-B12) were 0.74 · 10^{-5} cm/s and 0.45 · 10^{-5} cm/s, respectively. However, correction for the recruitment of perfused capillaries yields P_d(^{51}Cr-EDTA) = 1.5 · 10^{-5} cm/s and P_d(^{57}Co-B12) = 0.9 · 10^{-5} cm/s. The results indicate that the permeability of [illegible] muscle capillary for hydrophilic solutes is similar to that of continuous capillaries in other tissues. The Pappenheimer pore radius estimate of 30 Å, the Karnovsky [illegible] Å interendothelial slit width, and pinocytotic transport are defective in accounting for the results as ^{51}Cr-EDTA and ^{57}Co-B12 diffuse across the capillary membrane at rates proportional to their free diffusion coefficients in water (CDC(^{51}Cr-EDTA)/CDC(^{57}Co-B12) ≈ D(^{51}Cr-EDTA)/D(^{57}Co-B12) = 1.79). The transendothelial patent channel system of fused vesicles might be the pathway for transcapillary exchange of hydrophilic molecules as this system alone could account for the experimental results. Only 1–2 open channels per µm capillary length are necessary to account for the observed permeabilities. For both indicators CDC was a linear function of plasma flow and CDC increased 3–3.5 fold when plasma flow increased from [illegible] to 52 ml/100 g min. Assuming constant permeability coefficients, the CDC increase with plasma flow reflects an increase in capillary surface area indicating a 3–3.5 fold recruitment of capillaries. A constant ratio of CDC(^{51}Cr-EDTA)/CDC(^{57}Co-B12) within the perfusion range indicates that ^{51}Cr-EDTA and ^{57}Co-B12 can be employed at plasma flow [illegible] 4 ml/100 g min without risk of early back-diffusion resulting in underestimation of permeability.

Pappenheimer, Renkin and Borrero (1951) studied permeabilities for hydrophilic solutes of different molecular weights by the isogravimetric "osmotic transient" method. From analysis of the results in accordance with the theory of restricted diffusion (Faxén 1922, Ferry 1936) an equivalent pore radius estimate of 30 Å was obtained. The general theory based on 30 Å pores (Pappenheimer 1953) was seriously questioned by Crone (1963 b) who found that sucrose and inulin permeated skeletal muscle capillaries at rates proportional to their free diffusion coefficients in water. Paaske (1977 a and b) used ^{51}Cr-ethylenediamine-tetraacetate (^{51}Cr-EDTA) and ^{57}Co-cyanocobalamin (^{57}Co-B12) as indicators and obtained similar results for cutaneous and adipose tissues. These observations cannot be accounted for by the Pappenheimer pore radius dimension and it was doubted whether the

Supplements

[illegible]ood vessels and nerves were ligated and cut, and all visible fat was removed from the muscle. The thigh [illegible]uscles were separated from knee and distal part of femur. The crus was removed by disarticulation through [illegible] knee joint. The femur was plugged with cotton soaked in vaseline through a hole of 3 mm diameter [illegible]hich [illegible] drilled into the femur 2.0 cm proximal t[illegible] the knee joint. A screw was inserted to plug the hole. [illegible]he muscle [illegible] wrapped in moist gauze and covered with polyethylene sheets. The muscle was suspended [illegible]bove [illegible] copper plate that [illegible] heated by [illegible] ater thermostat to obtain [illegible] constant muscle temperature of [illegible]7°C. During suspension the length of the muscle corresponded to its normal resting length. Femoral artery [illegible]nd vein and sciatic nerve were isolated at the lower 4/5 of the thigh. All side branches of the vessels were [illegible] and cut except for one small arterial side branch. The sciatic nerve was ligated and cut so that [illegible]–6 cm segment was obtained leading to the gastrocnemius muscle. The femoral vein was cannulated as [illegible] as possible to the muscle as to allow collection of all effluent venous blood from the muscle. Blood [illegible] not recirculated. T[illegible] compensate for blood loss fresh whole blood of 37°C was given through [illegible] catheter [illegible] in the contralateral femoral vein. The contralateral femoral artery was catheterized to allow [illegible] pressure measurements via [illegible] Statham strain-gauge transducer [illegible] oscilloscope and [illegible] Varian or [illegible] [illegible]. The animal was heparinized (1 250 IE/kg) and 150 μg (30 μg/ml) unlabelled cyanocobalamin [illegible] injected via the femoral artery catheter [illegible]bout 5 min prior to the indicator injection. I[illegible] [illegible] experi[illegible] the sciatic nerve was brought in contact with silver electrodes connected to [illegible] stimulator. The blood [illegible] of the gastrocnemius muscle was adjusted by the stimulator to the desired constant blood flow value [illegible] individual expt. The [illegible]idth of the rectangular pulses was 0.1 ms and the voltage was 7 V. The pulse [illegible]requency was varied to obtain the desired blood flow (range 0.5–3 impulses/s) (Kjellmer 1964). The needle [illegible] external diameter 0.4 mm) of [illegible] micro-syringe was introduced into the small side branch of the femoral [illegible]artery and the tip of the needle [illegible] positioned in the femoral artery. A one inch NaI (Tl) scintillation detec[illegible]or (PW 4111 Type A, Philips, the Netherlands) was positioned above the muscle close to the surface. The [illegible]etector was collimated to see the muscle, exclusively. Using the micro-syringe [illegible] 5–7 μl bolus was injected [illegible] the femoral artery. The bolus contained 500 μCi ^{57}Co-cyanocobalamin (specific activity 150–300 μCi/μg; [illegible] Radiochemical Centre, Amersham, Great Britain) and 400 μCi ^{51}Cr-ethylene-diamine-tetraacetate [illegible] specific activity 40 mCi/mg; AG Hoechst, Germany). The needle [illegible] withdrawn [illegible] and removed immediately [illegible] after the bolus had been given. Impulses recorded in the area of registration were routed from the scintilla[illegible] detector to two interconnected [illegible] printing gamma spectrometers (Madotronic a/s, Denmark). The signals were pulse-height analysed with two individual [illegible] windows of acceptance adjusted around the [illegible] 320 MeV photopeak of ^{51}Cr and the 0.122 MeV photopeak of ^{57}Co, respectively. Recorded counts [illegible] [illegible] cross-talk [illegible] printed out [illegible] [illegible] loss each [illegible] and were stored on [illegible] paper tape [illegible] an interface [illegible] and [illegible] tape puncher (Type 4070, Facit AB, Sweden). Activity was recorded for 300 s. Effluent [illegible] blood from the muscle was collected, and volume per time unit and haematocrit value were determined. The gastrocnemius muscle was removed and weighed. Activity remaining in the field was determined, corrected [illegible] for cross-talk, and used as [illegible] background. The data of the paper tape were fed into [illegible] process computer [illegible] (Wang 600, Wang Corp. USA) via an electronic high-speed reader (Type 4022, Facit AB, Sweden).

Calculations

The computer [illegible] programmed to cross-talk correct ^{57}Co activities, to subtract the cross-talk corrected background values, and to perform kinetic analyses. Cross-talk of ^{57}Co to the ^{51}Cr channel was neglected ([illegible] 0.001). For each indicator the corrected count values were plotted as [illegible] function of time in [illegible] semilogarith[illegible] diagram by [illegible] Type 602 plotter-printer (Wang Corp. USA) connected to the computer.

Kinetic analysis of the recorded curve function for each indicator was performed in accordance with the principles originally described by Sejrsen (1970) using the computerized technique of Paaske and Nielsen (1976). The count value of the injected dose of indicator was taken as the highest recorded [illegible]alue. Peak time [illegible] the time after the bolus injection at [illegible]hich the maximum count value occurred. The part of the curve recorded in [illegible] interval as indicated in T[illegible]ble II (Extrapol. interval) was monoexponentially extra[illegible]polated to peak [illegible] using the "least square" regression procedure. The numerical value of this function [illegible] solved for each [illegible] was subtracted from the originally recorded [illegible]alue to gi[illegible] [illegible] intravascular transit function. Intravascular mean transit time was computed as $\bar{t}(iv)$ [illegible] area/height (Zierler 1965). Plasma flow [illegible] was calculated as f(pl) [illegible] (1/$\bar{t}$(iv)) [illegible] λ [illegible] 100 (ml/100 g [illegible] min) (Kety 1951). λ denotes intravascular plasma [illegible] [illegible] is estimated at 0.03 [illegible] (1 — Hct) (ml/g) [illegible]here Hct is hematocrit [illegible]alue (Sejrsen and Tønnesen, [illegible]personal communication). The value of the monoexponentially extrapolated curve at peak time [illegible] fraction of maximum count [illegible]alue gi[illegible] capillary extraction, E. The capillary diffusion capac[illegible]

Fig. 1 The autoperfused cat gastrocnemius preparation. For details, see text.

Karnovsky (1967 1968) interendothelial 40 Å slit of continuous capillaries (Bennett Luft and Hampton 1959 Majno 1965) was the morphological pathway for transcapillary exchange of hydrophilic solutes.

Krogh (1919) observed a 100 fold increase in number of perfused capillaries per unit cross sectional area of skeletal muscle during exercise as compared to resting state This order of magnitude for the "recruitment" phenomenon has been questioned by subsequent quantitative determinations (Landis and Pappenheimer 1963), and the general problem of recruitment or non-recruitment of capillaries with increased perfusion remains a debated subject (Crone 1975) Assuming constant permeability coefficients, determination of capillary diffusion capacity CDC (the permeability surface area product of Renkin) at different perfusion rates yields an opportunity to estimate the extent of increase in the surface of perfused permeable microvessels (capillaries) and thus possibly their number with increasing blood flow rates.

The present study aims at simultaneous determinations of CDC for -Cr EDTA and Co-B12 for the capillary membrane in skeletal muscle within a wide range of perfusion

Experimental procedure

E periments were perf rmed 16 cats of either sex weighing 3 .5 kg in mean (range 7 t 4 0 kg) After 12 h of fast ng anaesthesia was oduced by intraperitoneally jected chloralose (70 mg/kg). Tracheostomy was made, and n endotracheal t be was I troduced t flow free pontaneous respiration Rectal temperature was kept at 37 C usi g contact thermometer positioned n the rect m t con ol the temperature of heated operating t ble An toperfused gastrocnemius muscle preparation w made using the proced re applied by T nnesen d Sejrsen (1967) w th minor modification (F g. 1) The k of the hind limb from nguinal ligament to paw was emoved by thermoca ery and the Achilles tend w cut close t the calcaneus. The gastrocnemius muscle was isolated from surrounding tissues by blunt d section All

blood vessels and nerves were ligated and cut, and all visible fat was removed from the muscle. The thigh muscles were separated from knee and distal part of femur. The crus was removed by desarticulation through the knee joint. The femur was plugged with cotton soaked in vaseline through a hole of 3 mm diameter which was drilled into the femur 2.0 cm proximal to the knee joint. A screw was inserted to plug the hole. The muscle was wrapped in moist gauze and covered with polyethylene sheets. The muscle was suspended above a copper plate that was heated by a water thermostat to obtain a constant muscle temperature of 37°C. During suspension the length of the muscle corresponded to its normal resting length. Femoral artery and vein and sciatic nerve were isolated at the lower 4/5 of the thigh. All side branches of the vessels were isolated and cut except for one small arterial side branch. The sciatic nerve was ligated and cut so that a 4–6 cm segment was obtained leading to the gastrocnemius muscle. The femoral vein was cannulated as close as possible to the muscle as to allow collection of all effluent venous blood from the muscle. Blood was not recirculated. To compensate for blood loss fresh whole blood of 37°C was given through a catheter positioned in the contralateral femoral vein. The contralateral femoral artery was catheterized to allow blood pressure measurements on a Statham strain-gauge transducer, an oscilloscope and a Varian or Bennett writer. The animal was heparinized (1 250 IE/kg) and 150 μg (30 μg/ml) unlabelled cyanocobalamine was injected via the femoral artery catheter about 5 min prior to the indicator injection. In exercise experiments the sciatic nerve was brought in contact with silver electrodes connected to a stimulator. The blood flow of the gastrocnemius muscle was adjusted by the stimulator to the desired constant blood flow value of an individual expt. The width of the rectangular pulses was 0.1 ms and the voltage was 7 V. The pulse frequency was varied to obtain the desired blood flow (range 0.5–3 impulses/s) (Kjellmer 1964). The needle (internal diameter 0.4 mm) of a micro-syringe was introduced into the small side branch of the femoral artery and the tip of the needle was positioned in the femoral artery. A one inch NaI (Tl) scintillation detector (PW 4111, Type A, Philips, the Netherlands) was positioned above the muscle close to the surface. The detector was collimated to see the muscle, exclusively. Using the micro-syringe a 5–7 μl bolus was injected into the femoral artery. The bolus contained 500 μCi ^{57}Co-cyanocobalamin (specific activity 150–300 μCi/μg; the Radiochemical Centre, Amersham, Great Britain) and 400 μCi ^{51}Cr-ethylene-diamine-tetraacetate (specific activity 40 mCi/mg, AG Hoechst, Germany). The needle was withdrawn and removed immediately after the bolus had been given. Impulses recorded in the area of registration were routed from the scintillation detector to two interconnected universal printing gamma spectrometers (Meditronic a/s, Denmark). The signals were pulse-height analysed with two individual windows of acceptance adjusted around the 0.320 MeV photopeak of ^{51}Cr and the 0.122 MeV photopeak of ^{57}Co, respectively. Recorded counts including cross-talk were printed out without time loss each second and were stored on a paper tape via an interface unit and a tape puncher (Type 4070, Facit AB, Sweden). Activity was recorded for 300 s. Effluent venous blood from the muscle was collected, and volume per time unit and hematocrit value were determined. The gastrocnemius muscle was removed and weighed. Activity remaining in the field was determined, corrected for cross-talk, and used as background. The data of the paper tape were fed into a process computer (Wang 600, Wang Corp. USA) via an electronic high-speed reader (Type 4022, Facit AB, Sweden).

Calculations

The computer was programmed to cross-talk correct ^{57}Co activities, to subtract the cross-talk corrected background values, and to perform kinetic analyses. Cross-talk of ^{57}Co to the ^{51}Cr channel was neglected (< 0.001). For each indicator the corrected count values were plotted as a function of time in a semilogarithmic diagram by a Type 602 plotter/printer (Wang Corp. USA) connected to the computer.

Kinetic analysis of the recorded curve function for each indicator was performed in accordance with the principles originally described by Sejrsen (1970) using the computerized technique of Paaske and Nielsen (1976). The count value of the injected dose of indicator was taken as the highest recorded value. Peak time was the time after the bolus injection at which the maximum count value occurred. The part of the curve recorded in the interval as indicated in Table II (Extrapol. interval) was monoexponentially extrapolated to peak time using the "least squares" regression procedure. The numerical value of this function as solved for each second was subtracted from the originally recorded value to give the intravascular curve function. Intravascular mean transit time was computed as $\bar{t}(iv)$ = area/height (Zierler 1965). Plasma flow was calculated as f(pl) = (1/$\bar{t}$( )) · λ · 100 (ml/100 g · min) (Kety 1951). λ denotes intravascular plasma volume which is estimated = 0.03 · (1—Hct) (ml/g) where Hct is hematocrit value (Sejrsen and Tønnesen 1972 and personal communication). The value of the monoexponentially extrapolated curve at peak time expressed as a fraction of maximum count value gave capillary extraction, E. The capillary diffusion capac

TABLE I Experimental results. CDC(^{51}Cr EDTA) and CDC(^{57}Co-12) are presented in order of increas plasma flow which was determined from direct measurement of venous outflow The r CDC(^{51}Cr EDTA)/CDC(^{57}Co-B12) was in mean 1 79 which is identical to the value of D(^{51} EDTA)/D(^{57}Co-B12), *i.e* the ratio between the free diffusion coefficients in water at 37 Consequently Co-B12 is not subject to restricted diffusion as compared to ^{51}Cr EDTA in bed of continuous capillaries of skeletal muscle. CDC *for both indicators increase with increas* plasma flow indicating a 3-3.5 fold recruitment of capillaries.

Exp No	Plasma flow ml/100 g min	CDC(^{51}Cr EDTA) ml/100 g min	CDC(^{57}Co-B1) ml/100 g min	CDC(^{51}Cr EDTA)/ CDC(^{57}Co-B12)
1	4 0	3 8	1 6	2.38
2	5..	2.0	1 6	1..5
3	7 6	2.5	1 7	1 47
4	8.0	4.2	2.5	1 68
5	8.7	2.5	1.3	1 9
6	10.7	2.6	1 6	1 63
7	12.8	4 8	3 6	1.33
8	17.3	3 0	1 5	2.00
9	70.9	5.2	3 0	1 73
10	77 7	7 6	4 4	1 73
11	30.7	7 1	4.2	1 69
12	31 0	6.2	2.6	2.38
13	36.9	10 0	5.8	1 7?
14	45 9	8 9	5 7	1.56
15	52.1	7 9	3 7	2.14
16	52.4	9 1	4 4	2.07
$\bar{x}$	23.2	5 5	3 1	1 79
SE	4.2	0.7	0.4	0.08

ity CDC, defined as the unidirectional flux f indicator across the capillary membrane per 100 g tiss per unit concentration difference cross the capillary (Lassen and Trap-Jensen 1968 b) was calculated CDC = −f(pl) K ln (1 − E) (ml/100 g min) where K is a constant for converting ml of plasma to m of plasma ultrafiltrate. As K-values were used 0.89 for ^{51}Cr EDTA and 0.94 for ^{57}Co-B12 (Lassen an *Trap-Jensen 1968 b*).

Results

Table I tabulates CDC(^{51}Cr EDTA) and CDC(Co-B12) together with the plasma flo as measured directly from venous outflow Mean CDC(Cr EDTA) was 5 5 ml/100 g mi (S E. 0 7 ml/100 g min, n = 16), mean *CDC(Co-B12) was 3 1 ml/100 g min (S.E* 0 4 ml/100 g min), and mean f(pl) was 23 . ml/100 g min (S E. 4 2 ml/100 g min A linear plot of CDC(^{51}Cr EDTA) and CDC(Co-B12) as functions of f(pl) is presented i Fig. 2. CDC for both indicators increases linearly with increasing f(pl). As calculated fro the least square" regression method, the function CDC(^{51}Cr EDTA) *vs* f(pl) was satisfie by the straight *line equation* $y = 2.12 + 0.144x$ ($r = 0.90$, $p < 0.001$) This indicates tha CDC(^{51}Cr EDTA) increases from .7 to 9 7 ml/100 g min within the plasma flow rang from 4 to 52.4 ml/100 g min *i.e.*, with a factor of 3 6. For CDC(Co-B12) *vs* f(pl) $y = 1.43$ $0.071x$ ($r = 0.78$ $p < 0.001$) Thus, CDC(*Co-B12) increases from 1 7 to 5 2 ml/100 g mi* within the observed range, *i.e* with a factor 3 1 The ratio CDC(Cr EDTA)/CDC(Co-B12 was calculated for each experiment (Table I) Mean value was 1 79 (S E. 0 08) Fig. 3 presents CDC(^{51}Cr EDTA)/CDC(^{57}Co-B12) as function of f(pl) in a linear diagram. No

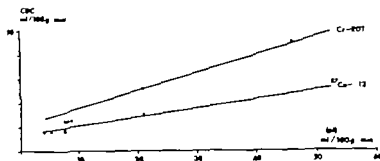


Fig. 2 The capillary diffusion capacity (CDC) of ^{51}Cr-ethylene-diaminetetraacetate (^{51}Cr EDTA) and ^{57}Co-cyanocobalamin (^{57}Co-B12) as determined simultaneously by the single injection, external registration method within the plasma flow range 4–52.4 ml/100 g min. The function CDC(^{51}Cr EDTA) vs. f(pl) obeys the straight line equation y = 2.12+0.144x. For CDC(^{57}Co-B12) vs. f(pl): y = 1.43+0.071x. Open circles ^{51}Cr-EDTA, filled circles ^{57}Co-B12

systematic deviation could be ascertained from the ratio between the free diffusion coefficients for the two indicators in water at 37°C, D(^{51}Cr-EDTA)/D(^{57}Co-B12) = 1.79 (solid line). By regression analysis y = 1.66 − 0.006x (r = 0.29 p > 0.1 95 per cent limits −0.20 to +0.69) indicating that significant correlation between the CDC ratio and plasma flow could not

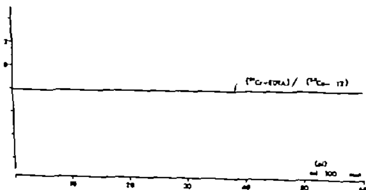


Fig. 3 The ratio CDC(^{51}Cr-EDTA)/CDC(^{57}Co-B12) plotted as function of plasma flow (f(pl)). No systematic deviation from the 1.79 value of the ratio between D(^{51}Cr EDTA)/D(^{57}Co-B12), the ratio between the free diffusion coefficients in water at 37°C, could be ascertained and there is no reason to believe that back-diffusion of the indicators occurs from interstitium to plasma

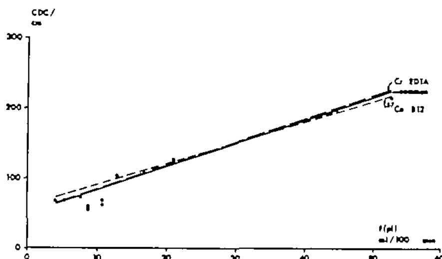


Fig. 4 The capillary diffusion capacity of each i dicator was normalized with respect to the proper free diffusion coefficient in water at 37°C. Absence of restricted diffusion across the continuous capillary membranes of skeletal muscle is demonstrated by the close greement of the regression lines. Consequently, transcapillary diffusion of the hydrophilic indicators takes place at rat constants proportional to the respective free diffuso coefficients. Open circles. ¹Cr EDTA filled circles Co-B12.

be demonstrated As restricted diffusion was not present for Co-B12 as compared to ¹Cr EDTA (CDC(Cr EDTA)/CDC(Co-B12) – D(Cr EDTA)/D(^{57}Co-B12) see discussion) Fig. 4 was constructed for CDC values normalized with respect to the respective D-values. CDC(Cr EDTA)/D(¹Cr EDTA) vs f(pl) yielded $y = 50.6 + 3.42x$ (r 0.90, $p < 0.001$) and CDC(Co-B12)/D(Co-B12) vs f(pl) gave y 61 1 + 3 02x (r 0 78, $p < 0.001$). The common CDC/D function of f(pl) gave $y = 49.0 + 3.41x$ ($r = 0.84$ p 0 001)

Mean capillary extraction of Cr EDTA E(Cr EDTA) was 0 282 (S E 0 031) and mean f(pl) as calculated from the Cr EDTA curve was 20 6 ml/100 g min (S E 3.5 ml/100 g min) Mean peak time for ¹Cr EDTA curves was 4 s (S E 1 s) Mean E(Co-B12) was 0 169 (S E 0 020) and a mean f(pl) of 23 9 ml/100 g min (S E 3 8 ml/100 g min) was calculated from Co-B12 curves. Peak time for Co-B12 was 4 s (S E 1 s) (Table II).

Significant differences could not be demonstrated between the directly measured plasma flow and plasma flow as determined by analysis of the indicator response curves (p 0 10, Wilcoxon s rank sum test for paired observations). The directly measured f(pl) and the f(pl) as calculated by kinetic analysis did not indicate any significant increase in vascular volume from rest to heavy exercise

The time interval chosen for monoexponential retropolation of the later part of the externally recorded curve is listed for each expt. Mean time for start of extrapolation was 8 97 f(iv) (S E 0.58 f(iv)) where f(iv) was calculated from the venous outflow Mean hematocrit value was 0 310 (S E 0 016) and the gastrocnemius muscle weighed in average 31 6 g (S.E 0 9 g).

The experimental tracings of expt No 9 is presented in Figs. 5 a and b

TABLE II. Experimental results. Capillary extraction, E, and plasma flow f(pl), as calculated by kinetic analysis of the residue curves. For explanation of Peak time and Extrapolation interval, see text. Hct denots hematocrit value.

Exp No	^{51}Cr EDTA E fraction	^{51}Cr EDTA f(pl) ml/100 g min	^{51}Cr EDTA Peak time	^{57}Co-B12 E fraction	^{57}Co-B12 f(pl) ml/100 g min	^{57}Co-B12 Peak time	Extrapol. Interval	Hct fraction	Muscle weight g
1	0.655	5.0	9	0.343	6.4	9	150–300	0.331	30.4
2	0.349	6.1	3	0.276	6.9	4	150–300	0.347	3[illegible].6
3	0.313	6.4	6	0. 15	7.5	5	150–300	0.446	39.4
4	0.443	6.0	7	0.279	9.3	5	150–300	0.277	29.9
5	0.276	12.2	6	0.147	15.4	6	150–300	0.359	79.4
6	0.[illegible]38	12.9	5	0.146	15.4	5	110–[illegible]90	0.26[illegible]	35.8
7	0.342	11.7	6	0.258	11.9	5	150–300	0.307	28.2
8	0.178	18	3	0.086	18.5	3	60–180	0.363	35.4
9	0.243	20.4	2	0.143	18.5	1	60–180	0.266	33.8
10	0.264	17.9	2	0.155	25.9		40–120	0.341	28.3
11	0.228	21	2	0.135	30.4	2	30–90	0.374	30.4
12	0.200	36.8	2	0.086	39.6	2	30–90	0.328	26.9
13	0.262	27.7		0.154	36.8	2	30–90	0. [illegible]40	25.5
14	0.195	36.1	2	0.124	41.5		25–75	0.26[illegible]	34.1
15	0.156	43.9	2	0.072	50.7	2	20–75	0.262	34.3
16	0.177	47.6	2	0.086	47.5	2	20–75	0.192	30.5
$\bar{x}$	0.282	20.6	4	0.169	23.9	4		0.310	31.6
SE	0.031	3.5	1	0.020	3.8	1		0.016	0.9

Discussion

I. *Methodological considerations*

Application of the single injection, external counting method to a single inlet, single outlet system has been discussed elsewhere (Sejrsen 1970, Paaske and Nielsen 1976) and only features of special significance for the present series will be considered.

As the residue curves follow multiexponential function within the first hour of registration after the bolus injection (Larsen and Sejrsen 1971) the time interval of monoexponential extrapolation was chosen in order to obtain minimum effect of the bending of the curve function together with a reasonable number of observations (count values). Support of the validity of this procedure is indicated by agreement between directly measured blood flow from the venous outflow of the muscle and values obtained by kinetic analysis of the externally recorded response curves for each of the two indicators.

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II. *Permeability data*

A. *General considerations.* The results indicate that CDC for both indicators increases linearly with plasma flow. Consequently it becomes essential that comparison between

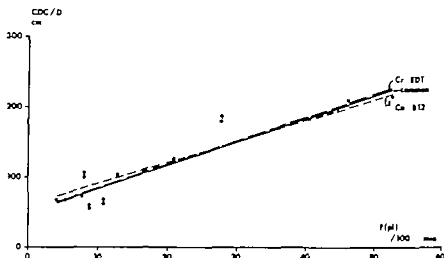


Fig. 4 The capillary diffusion capacity of each indicator was normalized with respect to the proper free diffusion coefficient in water at 37°C. Absence of restricted diffusion across the continuous capillary membranes of skeletal muscle is demonstrated by the close agreement of the regression lines. Consequently, transcapillary diffusion of the hydrophilic indicators takes place at rate constants proportional to the respective free diffusion coefficients. Open circles [51]Cr EDTA, filled circles [57]Co-B12.

be demonstrated. As restricted diffusion was not present for Co-B12 as compared to Cr EDTA (CDC([51]Cr EDTA)/CDC(Co-B12) – D([51]Cr EDTA)/D(Co-B12) see discussion) Fig. 4 was constructed for CDC values normalized with respect to the respective D-values. CDC(Cr EDTA)/D([51]Cr EDTA) or f(pl) yielded $y = 50.6 + 3.42x$ ($r = 0.90$, $p < 0.001$) and CDC(Co-B12)/D(Co-B12) or f(pl) gave $y = 61.1 + 3.02x$ ($r = 0.78$, $p < 0.001$). The common CDC/D function of f(pl) gave $y = 49.0 + 3.41x$ ($r = 0.84$, $p < 0.001$)

Mean capillary extraction of Cr EDTA E(Cr EDTA) was 0.282 (S.E. 0.031) and mean f(pl) as calculated from the [51]Cr EDTA curve was 20.6 ml/100 g min (S.E. 3.5 ml/100 g min) Mean peak time for Cr EDTA curves was 4 s (S.E. 1 s) Mean E(Co-B12) was 0.169 (S.E. 0.020) and a mean f(pl) of 23.9 ml/100 g min (S.E. 3.8 ml/100 g min) was calculated from Co-B12 curves. Peak time for Co-B12 was 4 s (S.E. 1 s) (Table II).

Significant differences could not be demonstrated between the directly measured plasma flow and plasma flow as determined by analysis of the indicator response curves ($p > 0.10$, Wilcoxon's rank sum test for paired observations) The directly measured f(pl) and the f(pl) as calculated by kinetic analysis did not indicate any significant increase in vascular volume from rest to heavy exercise

The time interval chosen for monoexponential retropolation of the later part of the externally recorded curve is listed for each expt. Mean time for start of extrapolation was 8.97 $\bar{t}$(iv) (S.E. 0.58 $\bar{t}$(iv)) where $\bar{t}$(iv) was calculated from the venous outflow Mean hematocrit value was 0.310 (S.E. 0.016) and the gastrocnemius muscle weighed in average 31.6 g (S.E. 0.9 g)

The experimental tracings of expt No 9 is presented in Figs. 5 a and b.

TABLE II. Experimental results. Capillary extraction, E, and plasma flow f(pl), as calculated by kinetic analysis of the residue curves. For explanation of Peak time and Extrapolation interval, see text. Hct denotes hematocrit value.

Exp No	^{51}Cr-EDTA			^{57}Co-B$_1$			Extrapol. interval	Hct fraction	Muscle weight g
	E fraction	f(pl) ml/100 g min	Peak time	E fraction	f(pl) ml/100 g min	Peak time			
1	0.435	5.0	9	0.343	6.4	9	150–300	0.331	30.4
2	0.349	6.1	3	0.276	6.9	4	150–300	0.347	32.6
3	0.313	6.4	6	0.215	7.5	5	150–300	0.446	39.4
4	0.443	6.0	7	0.279	9.3	5	150–300	0.277	29.9
5	0.276	12.2	6	0.147	15.4	6	150–300	0.359	29.4
6	0.238	12.9	5	0.146	15.4	5	110–250	0.262	35.8
7	0.342	11.7	6	0.238	11.9	5	150–300	0.307	28.2
8	0.176	18.2	3	0.086	18.5	3	60–180	0.363	35.4
9	0.243	20.4	2	0.143	18.5	1	60–180	0.266	33.8
10	0.264	17.9		0.153	23.9	2	40–170	0.341	28.3
11	0.228	21.2	2	0.135	30.4		30–90	0.374	30.4
12	0.200	36.8	2	0.086	39.6	2	30–90	0.328	26.9
13	0.262	27.7	2	0.154	36.8	2	30–90	0.240	23.5
14	0.195	36.1	2	0.1[illegible]4	41.5	2	25–75	0.262	34.1
15	0.156	43.9	2	0.072	50.7	2	20–75	0.282	34.3
16	0.177	47.6	2	0.084	47.5	2	20–75	0.192	30.5
	0.282	20.6	4	0.169	23.9	4		0.310	31.6
SE	0.031	3.5	1	0.020	3.8	1		0.016	0.9

Discussion

I. *Methodological considerations*

Application of the single injection, external counting method to a single inlet, single outlet system has been discussed elsewhere (Sejrsen 1970, Paaske and Nielsen 1976) and only features of special significance for the present series will be considered.

As the residue curves follow a multiexponential function within the first hour of registration after the bolus injection (Larsen and Sejrsen 1971) the time interval of monoexponential extrapolation was chosen in order to obtain minimum effect of the bending of the curve function together with a reasonable number of observations (count values). Support of the validity of this procedure is indicated by agreement between directly measured blood flow from the venous outflow of the muscle and values obtained by kinetic analysis of the externally recorded response curves for each of the two indicators.

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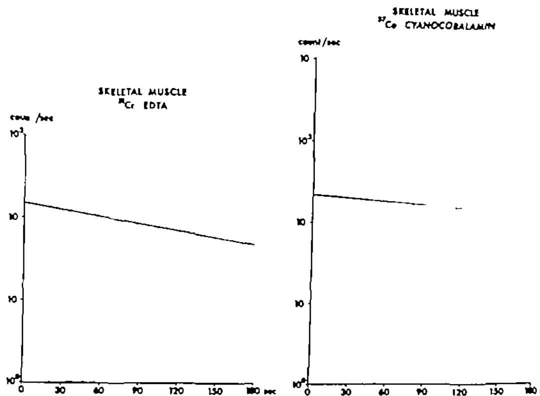


Fig. 5. Fig. 5 a presents the computer printout of the recorded count alues for ⁵¹Cr EDTA corrected fo background activity (9 cps) plotted as a function of time in a semilogarithmic diagram (dots) (E pt. No. 9 At time zero the bolus i jection was given A maximum count value of 617 cps was reached at 2nd s (pea time). The part of the curve recorded in the time nterval 60 s–180 s obeyed the equation cps(t) 151 exp (−0.0063 t) where t is time in s. The standard deviation on the slope SD(k), was 0 0003 s⁻¹ At pea time (2nd s) a value of 150 was obtained Capillary extraction E(⁵¹Cr EDTA), was 150/617 = 0 43 Plasm flow as measured directly was 20 9 ml/100 g min and CDC(⁵¹Cr EDTA) = 20 9 0 89 ln (1 0 4 (ml/100 g min) = 5.2 ml/100 g min. As the mean transit time of ⁵¹Cr EDTA plasma as 0 108 m (crosses denote the vascular transit) the plasma flow could be calculated from f(pl) = (1/0.108) (1 0 766) 0 03 100 (ml/100 g min) = 20.4 ml/100 g min

The simultaneously recorded ⁵⁷Co-B12 traci g given in F g. 5 b, where count values corrected fo cross-t lk and backgrou d activ ty are plotted as function of time in a semilogarithmic diagram Cross tal correction factor from ⁵¹Cr to Co was 0.48 Background alue fo Co-B12 was 22 cps. Maxi mum cou t alue, 1 510 cps, was reached at 1st The extrapolation slope was gi en by cps(t) 215 exp (−0 0030 t) with SD(k) = 0.0003 s⁻ E(⁵⁷Co-B12) wa 15/1 510 = 0 143 gi ing CDC(⁵ Co-B12) 3 0 ml/100 g m n As determined from kinetic nalysis of the ⁵⁷Co-B12 curve plasma flow was 18 ml/100 g m *The ratio CDC(⁵¹Cr EDTA)/CDC(Co-B12) was 1 71*

CDC values for a given indicator is performed between values obtained at identical plasma flows. Table III summarizes literature values of CDC for sucrose and Cr EDTA which *for all practical purposes have same diffusion and permeability characteristics. Good* agreement with the results of the present series is seen, when account is taken for the plasma flows. In "hyperemic" skeletal muscle in man CDC(Co-B12) was 1 7 ml/100 g min and CDC(Cr EDTA) was 3 4 ml/100 g min (Lassen and Trap-Jensen 1968 a) These values correspond to the results of the present series. At a plasma flow of 7 ml/100 g min CDC(Co-B12) can be calculated to 1 9 ml/100 g min from the equation y 1 43 0 071x. At S = 70 cm /g and f(pl) 7 ml/100 g min, P_d(Co-B1) becomes 0 45 10

TABLE III. Capillary diffusion capacity, CDC, and permeability coefficient, P_d, for sucrose and ^{51}Cr EDTA in skeletal muscle as measured by methods based on indicator diffusion.

Indicator	CDC ml/100 g min mean (range)	P_d 10^{-5} cm/s mean	Plasma flow f(pl) ml/100 g min mean (range)	Method	Species & Tissue	Reference
Sucrose	3.1	0.74	~ 7.2	ID	Dog hindlimb	Crone 1963
^{51}Cr EDTA	{4.8 (3.5–5.8); 4.4 (3.5–6.1)}	{1.14; 1.04}	14.7 (11.6–23.3)	{ID; SI}	Cat gastroc. nemius	Sejrsen 1970 and pers. comm.
Sucrose/ ^{51}Cr EDTA	3.6 (2.3–4.7)	0.86	14.1 (6–26)	ID	Human forearm	Trap-Jensen and Lassen 1970
^{51}Cr-EDTA	3.5 (2.–4.9)	0.83	18.9 (10–31)	TC	Human ant. tib. m.	Trap-Jensen et al. 1970
^{51}Cr EDTA	3.2 (1.5–4.5)	0.77	~ 14 (4.4–23.4)	ID	Human forearm	Trap-Jensen and Lassen 1971
^{51}Cr-EDTA	5.5 (2.0–10.0)	{0.74 at f(pl) ~ 7; 1.02 at f(pl) 15; 1.30 at f(pl) ~ 23.2}	23 (4.0–52.4)	SI	Cat gastroc. nemius	Paaske 1977 (present study)

Abbreviations. ID: indicator diffusion method, SI: single injection, external counting method, TC: tissue clearance method.

Note. All P_d-values are calculated at a capillary surface area, S, of 70 cm^2/g. From the arguments presented in the discussion, S must be considered to increase with increasing plasma flow. Normalizing with respect to this phenomenon, P becomes ~ 1.3 × 10^{-5} cm/s for the present series. A capillary surface area of 35 cm^2/g is probably a better estimate at a plasma flow of 7 ml/100 g min.

EDTA and ^{57}Co-B12 are in agreement with results obtained in other tissues with capillaries of continuous type (Paaske 1977 a). The results support the hypothesis suggested previously that capillaries of continuous type exhibit same permeation characteristics regardless of the tissue in which they are located (Paaske 1976).

B. *Capillary pore size.* The ratio between the free diffusion coefficients in water at 37°C is D(^{51}Cr EDTA)/D(^{57}Co-B12) = 0.700 × 10^{-4} (cm/s)/0.390 × 10^{-4} (cm/s) = 1.79 (Paaske 1977 a). In the present series CDC(^{51}Cr EDTA)/CDC(^{57}Co-B12) ~ 1.79 which implies that the diffusion of these indicators across the capillary membrane of skeletal muscle takes place with rate constants proportional to the free diffusion coefficients of the two indicators. The data can therefore obviously not be applied for calculation of an equivalent pore radius. The concept of a circular and cylindrical water-filled pore of 30 Å radius or—alternatively—a slit width of 37 Å was introduced by Pappenheimer, Renkin and Borrero (1951). Applying the isogravimetric osmotic transient method to the cat hindlimb preparation it was found that the permeability coefficients for a series of indicators decreased more than could be anticipated from the decrease in free diffusion coefficients. From analysis employing the theory of restricted diffusion (Faxén 1922, Ferry 1936) the 30 Å pore radius estimate was obtained. This estimate was questioned by Crone (1963 b) who applied the "indicator diffusion" method (Crone 1963 a) to the dog hindlimb preparation. This technique is not complicated by osmotic reflection coefficient corrections and the finding of absence of restricted diffusion to inulin as compared to sucrose leads to a considerably larger pore radius estimate.

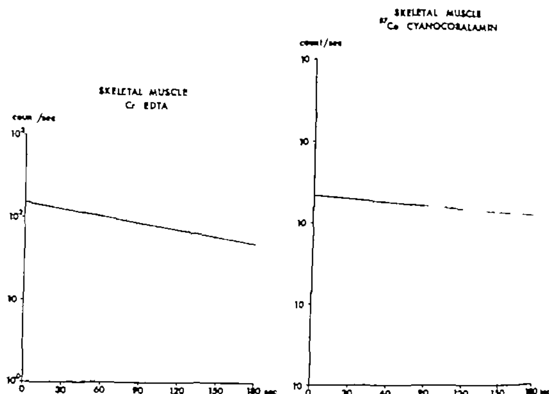


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By an alternative indicator diffusion technique this finding has recently been confirmed (Paaske and Sejrsen 1977) From studies based on filtration expts. pore radius estimates from 31 to 45 Å have been obtained (Grotte 1956, Renkin and Pappenheimer 1957 Landis and Pappenheimer 1963 Diana, Long and Yao 1972) whereas studies of diffusion permeability (Crone 1963 b Lassen and Trap-Jensen 1968 a, Paaske and Sejrsen 1977 present study) showed absence of restricted diffusion for molecules in the molecular weight range 340–5 500. Yet, Trap-Jensen and Lassen (1970, 1971) found massive restriction for molecules in the same range by the "indicator diffusion method applied to the human forearm (slit width estimate 40 Å)

The findings of the present study are incompatible with the belief that transcapillary exchange of hydrophilic solutes in skeletal muscle takes place through the Karnovsky 40 Å interendothelial slit which has been speculated to be morphological equivalent for the Pappenheimer pore (Karnovsky 1967 1968) However in a recent study by Simionescu, Simionescu and Palade (1975) a channel system of fused intraendothelial vesicles was demonstrated by electron micrographs in the continuous capillary of skeletal muscle. Although the significance of this system is not established as yet it appears that transendothelial passage of lipid-insoluble molecules through such a system is compatible with permeability data obtained in a number of physiological studies (Kruhoffer 1946 Crone 1963 b, Lassen and Trap-Jensen 1968 a, Alvarez and Yudilevich 1969 Boyd *et al* 1969 Normand *et al* 1971 Bassingthwaighte, Yipintsoi and Grabowski 1975 Paaske 1977 a and b, Paaske and Sejrsen 1977) Considering the compiled data from studies based on indicator diffusion it would seem reasonable to believe that the equivalent pore radius is at least 90 Å Recent data from lung (Perl Chowdhury and Chinard 1975) and frog mesentery (Curry Mason and Michel 1976) have questioned the belief that the magnitude of the osmotic reflection coefficient increases with increasing molecular size of the test indicator for these continuous capillary membranes. Considering the experimental errors in the studies mentioned above these findings might be accounted for by assuming a larger pore than is conventionally considered based on measurements of filtration. The lower pore radius estimate of filtration studies might therefore be defective due to incomplete experimental knowledge of the reflection coefficients and the possibility that an alternative pathway for water filtration exists in addition to the pathway for the hydrophilic solutes. These possibilities might contribute to the *"Pappenheimer pore puzzle" as Perl s (1971) solution to this problem was based on a* 40 Å slit width and becomes defective with a pore radius so large as it does not exhibit significant restricted diffusion to Co-B12 compared with ^{51}Cr EDTA The present data also exclude the necessity for a large pore system and vesicular transport of these hydrophilic molecules is excluded since their transport takes place with rate constants proportional to the free diffusion coefficients.

Assuming a pore radius of 90 Å the pore area, S_p, can be calculated as S CDC Δx_d/D (cm /g) where Δx_d is diffusion distance across the endothelium As the "patent channel" system seems to be located perpendicularly to the basement membrane we consider $\Delta x_d \sim 1\,000$ Å. At CDC(^{51}Cr EDTA) = 3 7 ml/100 g min S = ((3 7/100 60) 1 10)/ 0 7 10^{-5} = 8 8 10^{-4} cm /g. At S = 70 cm /g we obtain S_p/S = 1.26 10 *i e* the pore area available for transendothelial diffusion is ~ 1/100 000 of the total capillary surface area.

The number of pores per g tissue which at any time must be open is $S_p/\pi r^2 = 8.8 \cdot 10^{-4}/2.54 \cdot 10^{-14} = 3.5 \cdot 10^{9}$. Approximately 175 capillaries are open in 1 mm² of skeletal muscular tissue at a plasma flow corresponding to the chosen CDC(⁵¹Cr EDTA) (Landis and Pappenheimer 1963). This leads to $3.5 \cdot 10^{9}/1.75 \cdot 10^{5} = 2 \cdot 10^{4}$ open pores per capillary. At a capillary length of 1 200 μm we get an estimate of ~1.2 open pores per μm capillary length. This number could very well explain the fact that channels of fused vesicles are rarely seen on electron micrographs.

C. *Recruitment.* The present study indicates (Table I, Figs. 2 and 4) that CDC for the two indicators increases 3–3.5 fold from resting muscle (f(pl) = 4 ml/100 g · min) to heavily exercising muscle (f(pl) · 52 ml/100 g · min). Three possible explanations for this phenomenon can be considered: 1) constant capillary surface area with increased pore density times pore cross sectional area, 2) increased capillary surface area with constant pore density times pore cross sectional area, 3) increased capillary surface area with increased pore density times pore cross sectional area. As permeability coefficient and surface area are inseparable in their product no conclusive statement as regarding the validity of either explanation can be obtained from the present data. From studies on the capillary filtration coefficient, CFC, of the Starling formulation of transcapillary fluid movement, it is known that CFC increases 3–4 fold with transition from resting to exercising state in hind part in cats (Cobbold *et al.* 1963) and in cat calf muscles (Kjellmer 1964). As argued by Kjellmer (1964) CFC should increase in proportion t· the 4th power of the pore radius (Poiseuille's law) while CDC for hydrophilic solutes would increase with the 2nd power (Fick· law), if an increase in pore size · as responsible. At · CDC increase of factor 3 CFC should consequently increase 9 fold. However, if capillary surface area is responsible CFC should increase 3 fold. Considering the CFC data and this argumentation it would seem reasonable to assume that the CDC and CFC increases should be attributed to an increased capillary surface area rather than an increase in pore size. From histological studies it is known that the number of open capillaries per unit cross sectional area of skeletal muscle varies (Landis and Pappenheimer 1963, Hammersen 1968). A part of the blood to tissue exchange area might be positioned in venules. For this reason, it is doubtful whether CDC and CFC data can be correlated rigorously to capillary counts.

By the criticized (Crone 1970) continuous infusion method Renkin, Hudlická and Sheehan (1966) obtained results indicating that CDC for ⁸⁶Rb and ⁴²K increased 2.5 fold in the flow range from 7.9 t· 30.2 ml/100 g · min in canine gracilis muscle. An increase of CDC for hydrophilic indicators with increasing plasma flow has not been demonstrated in other studies in skeletal muscle. This is probably due to the fact that all other comparable studies (Table III) have operated at plasma flows of 31 ml/100 g · min or lower. In other tissues CDC for various indicators has recently been shown to be linearly related to plasma flow ·e. myocardium (L'Abbate *et al.* 1976), lung (Basset *et al.* 1974), and submandibular gland (Mann, Smaje and Yudilevich 1976).

Assuming as above that CDC is linearly related to capillary surface area, S, and assuming capillary radius 3.2 μm, the ordinate axis on Fig. 4 can be considered to provide an estimate of surface area of perfused capillaries in cm²/g by dividing the ordinate axis values by two. The consequences of these considerations are that · capillary surface area of ~35 cm²/g

would probably be a more correct estimate for plasma flows ~7 ml/100 g min leading to P_d-values of ~1.5 10⁻ cm/s and ~0 9 10^{-4} cm/s for ¹Cr EDTA and ⁵⁷Co-B12, respectively Further it can be calculated that a capillary volume of ~12 per cent of total vascular volume is present in resting condition whereas in maximum exercise ~50 per cent of the vascular volume will be located in exchange vessels. These considerations are in agreement with Landis and Pappenheimer (1963). This has not been taken into account with the kinetic calculation of plasma flow where a vascular volume of 0 03 ml/g has been used in all instances *as a reasonable estimate. This was based on the fact that the experiments did not provide* evidence for an increase of *total* intravascular volume. The intermittent mechanical compression of the vessels during exercise might influence an eventual redistribution of blood volume within the vascular system of the muscle.

The mean transit time for the indicators in the capillaries can be calculated from $\bar{t}$(cap) V(cap)/f(pl), where V(cap) is intracapillary plasma volume. Assuming V(cap) at rest to be 12 per cent of the total intravascular volume, the mean transit time at resting perfusion (4 ml/100 g min) becomes $\bar{t}$(cap-rest) = 0 03 0.12 (1 − Hct) 100 60/4 ~ 4 s. During maximum exercise (f(pl) = 52 ml/100 g min) V(cap) is increased with the factor 3–3.5. Consequently $\bar{t}$(cap-exercise) = 0 03 0.50 (1 − Hct) 100 60/52 ~ 1 2 s. The linear velocity of the blood will increase with a factor of $\bar{t}$(cap-rest)/$\bar{t}$(cap-exercise) = 4/1.2 ~ 3.5 When the number of functioning capillaries is increased with a factor 4 from rest to maximum exercise this will reduce the intercapillary distances with a factor 2, resulting in a reduction in diffusion time with a factor 4 This reduction in diffusion time will compensate for the 3.5 fold increase in linear velocity

D *Back-diffusion.* Back-diffusion of indicator is defined as early reentry of indicator from interstitial fluid to blood while the indicator bolus is still in exchange vessels. Capillary permeability will consequently be underestimated. In the present series significant positive correlation could not be demonstrated between CDC(¹Cr EDTA)/CDC(Co-B12) and plasma flow *i e* there is no reason to believe that back-diffusion occurs to a significant extent for the employed indicators within the observed plasma flow range of 4–52 ml/100 g min (Fig. 3), and no experimental validation was found for the assumption (Schafer and Johnson 1964) that extraction of ¹Cr EDTA (and Co-B12) should be blood flow limited. This finding is of importance for interpretation of measured CDC(¹Cr EDTA) and CDC(Co-B12) values obtained in the continuous capillary bed of cutaneous and adipose tissues in rabbits in which the physiological plasma flow range is from 4 to 10 ml/100 g min (Paaske and Nielsen 1976, Paaske 1976, 1977 a and b) On this basis it is unlikely that these results are influenced by back-diffusion In adipose tissue which has about half the capillary surface area of skeletal muscle it should be possible to perform CDC determinations with these indicators without back-diffusion even at plasma flows of some 2 ml/100 g min. The position of individual patent channels is probably a stochastic variable. If the dynamic processes of formation and degradation of vesicles are sufficiently fast it would seem improbable that an individual molecule will pass from interstitium to blood *via* the same pathway through which it entered the interstitium. The resulting increase in interstitial diffusion distance will reduce the probability for back-diffusion while the bolus is still located within exchange vessels.

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The Effect of Indomethacin on Renal Blood Flow Distribution during Hemorrhagic Hypotension in Dog

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Abstract

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The effect of indomethacin in hemorrhagic hypotension (HH) on total renal blood flow (RBF) and cortical flow distribution was studied in 9 pentobarbital anesthetized dogs. Local blood flow was measured as hydrogen clearance by 6 platinum electrodes in outer and inner cortex. RBF was recorded by electromagnetic flowmeter. Injection of indomethacin (3–5 mg/kg b.wt.) to inhibit prostaglandin synthesis, reduced renal blood flow only a few percent in control period. After the pretreatment with indomethacin, bleeding to mean arterial pressure 30 mmHg decreased RBF abruptly to 25 % of control flow. Thus renal vasoconstriction is observed both in early and late periods of bleeding in contrast to the initial vasodilation observed during the first half an hour of HH when prostaglandin formation is not blocked. Local blood flow in outer cortex decreased proportionally to flow in inner cortex during bleeding in both the indomethacin treated and in the untreated group, indicating an equal vasodilatory effect of prostaglandin in all parts of renal cortex during early periods of HH.

Several studies of renal hemodynamics in hemorrhagic hypotension (HH) have shown an initial reduction of the renal vascular resistance, followed by a marked increase of resistance in both cortex and medulla after 1/2–1 h (Jirka *et al.* 1961, Aukland and Wolgast 1968, Aukland *et al.* 1973, Kirkebø and Tyssebotn 1974). While Aukland and Wolgast (1968) proposed that this resistance fall might be a kind of autoregulatory response to the decreased blood pressure, it still remains to describe the mechanisms involved in such a response. Prostaglandin E (PGE) is a possible mediator as it was recently shown that the concentration of PGE in arterial blood is markedly increased during HH (Flynn *et al.* 1975, Johnston and Selkurt 1976). Since a number of studies have located the renal PGE synthesis to the outer medulla (Lee *et al.* 1967), vasodilation caused by endogeneous PGE would be expected to be limited to outer medulla and/or the deepest part of inner cortex. This would be compatible with findings of especially low flow in inner cortex after blockade of PGE synthesis with indomethacin during HH (Data *et al.* 1976), but does not fit with our findings of an unchanged cortical flow distribution during HH (Aukland *et al.* 1973, Kirkebø and Tysse-

botn 1974) It is possible, however that without any release of PGE, the vasoconstriction would have been even more marked in the inner cortex.

In view of these considerations we wanted to find out whether indomethacin blockade of PGE synthesis would alter the pattern of resistance change during HH—and if so, whether the effect would be limited to certain parts of the cortex.

All results in the present study are compared to results from a series of expts, made with identical procedure, but without use of any blockers (Aukland *et al* 1973)

Methods

Experiments were made on 9 mongrel dogs f both sexes, weighing 18–23.5 kg. Anesthesia was induced by pentobarbital 25 mg/kg b wt. and additional doses given when needed, usually bout 3 mg/kg h The dogs had free access to water but no food was given for 16 h prio to anesthesia.

Polyethylene catheters were placed in femoral artery and vein for bleeding nd infusions respectively A catheter was placed in brachial artery for pressure recording.

The kidney was exposed retroperitoneally through flank ncision and the renal artery gently dissected free. A polyvinyl catheter was sewn int the renal artery (Herd and Barger 1964) for upstream injection of H saturated saline heated to 37°C. An electro-magnetic flow probe was placed on the renal artery for total renal blood flow measurement (Nycotron) The flowmeter was calibrated directly femoral artery

L-shaped platinum electrodes were inserted to different depths of the cortex so that 3 had the sensitive electrode tip in outer half of the cortex, and 3 in the inner half The electrodes were 0.2 mm in diameter and made of 90 % platinum and 10 % iridium coated with an enamel lacquer The sensitive electrode tip was made by stripping off the lacquer from 0.5 mm of the electrode tip

H saturated saline was injected until constant current was measured polarographically on a *Rikadenki* recorder (Kogyo B64) t constant polarization potential of +0.2 V *vs* an Ag/AgCl electrode—then the injectio was suddenly stopped and the washout curve recorded The flow in ml/min g was determined from the formula K = ln 0.5/T½ where T½ in min is the halflif of the H_2 current (Aukland *et al.* 1964) All washo t curves were monoexponential down to 10 % f initial saturation when plotted on a semilogarithmic paper and T½ was easily determined

Control measurements were made the last 20 mi before bleeding was started. Indomethacin was given i.v in doses of 3–5 mg/kg b wt. bout 10–15 min before bleeding started, 2 3 measurements of RBF and local flow were made after indomethacin was given

Bleeding procedure

The dog was bled to a mean arterial pressure ($\overline{AP}$) *of* 50 mmHg 5–20 *min by free flow through the* catheter in the femoral artery into a reservoir placed 68 cm bove heart level. Heparin (5000 I U) was given into the reservoi H_2 washout curves were made with few min i tervals in the first half hour of bleedi g, later ith intervals f bout 20 min, until retransfusion started 2 h after the in tiation f bleedi g. Reinfusion of the heparinized reservo r *blood induced profuse bleeding* i 6 *dogs, making measurements after retransfusion* unappropriate

At the end of the expt. the kidney was excised and the pos tion of each electrode caref lly examined All electrodes were located in the right cortical half d no electrodes were excluded The drained kidney weight was used f calculation of RBF i ml/mi g.

The present measurements are compared to results a previously published study (7 expts) (A kland *et al* 1973) where the surgical nd bleeding procedure as well as technical equipment were identical

Using paired t-tests f r statistical evaluation, differences were considered significant t p 0 05

Results

Control measurements

The average mean arterial pressure ($\overline{AP}$) was 124 mmHg, with a range of 110 to 140 mmHg. ... blood flow (RBF) averaged 3.50 (±S D 0 79) ml/min At the same

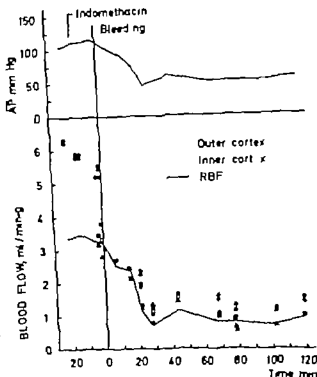


Fig. 1. Effect of indomethacin and bleeding on mean arterial pressure ($\overline{AP}$), total (RBF) and local blood flow at 6 separate electrode sites in outer and inner cortex of one dog

the average outer cortical blood flow (OCF) and inner cortical blood flow (ICF) were 3.57 (± S.D. 0.83) and 3.43 (± S.D. 0.60) ml/min · g respectively. This gives a ratio OCF/ICF of 1.04 which is not significantly different from unity.

After one i.v. injection of indomethacin 3–5 mg/kg b.wt. $\overline{AP}$ increased only 3.1% from control level whereas RBF decreased 10.2% ($p < 0.05$). Total renal vascular resistance increased 14.8% ($p < 0.02$). Local blood flow and its distribution changed little as OCF and ICF decreased 6.1% and 6.0% respectively (OCF/ICF ratio 1.04). These data indicate a slight rise in vascular resistance in all cortical layers in the kidney. The absolute flow values agreed well with control values in a previous study (Aukland *et al.* 1973) finding that RBF, OCF and ICF were 5.30, 3.47 (± S.D. 0.83) and 3.61 (± S.D. 0.86) ml/min · g respectively. Average $\overline{AP}$ was 118 mmHg.

Hemorrhagic hypotension

All dogs were bled 900–1 000 ml to reach an $\overline{AP}$ of 50 mmHg within 20 min, and this blood pressure level was maintained for about 2 h before beginning retransfusion of shed blood. H_2 clearances were measured every 5–10 min in the first half hour of bleeding, later every 20 min.

RBF fell rapidly during bleeding, even faster than the fall in $\overline{AP}$. Local blood flow at all electrode sites in outer and inner cortex fell simultaneously and to the same degree as the RBF as shown for a single experiment in Fig. 1.

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In view of these considerations we wanted to find out whether indomethacin blockade of PGE synthesis would alter the pattern of resistance change during HH—and if so, whether the effect would be limited to certain parts of the cortex.

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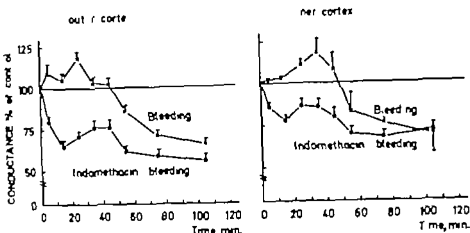


Fig 3 Relative vascular conductance (±S E.) in outer and inner renal cortex during bleeding (n 7) (crosses) and during bleeding after pretreatment with indomethacin (n 9) (open circles)

RBF respectively after injection of indomethacin in pentobarbital anesthetized dogs. In conscious dogs, however Swain *et al.* (1975) found only 12 % decrease in renal blood flow while renal resistance increased 15% after indomethacin treatment in accordance with our results on anesthetized dogs, suggesting that the effect of the anesthetization and the handling of the kidney is minimal in our study

Total renal blood flow during bleeding The renal response to hemorrhage has classically been described as a vasoconstriction (Trueta *et al.* 1947) whilst more recently published studies have shown that even a vasodilation may occur especially in the first period of HH (Ivka *et al.* 1961 Aukland and Wolgast 1968, Kirkebo and Tyssebotn 1974). In our previous study (Aukland *et al.* 1973) using identical experimental procedure as in the present experiments, a fall in vascular resistance was observed during the first 30 min of bleeding, followed steadily increase in resistance, probably caused by an initial autoregulatory response which gradually is overpowered by increasing vasoconstrictor activity

According to several investigators (Anderson *et al.* 1975, Venuto *et al.* 1975), however indomethacin does not influence renal autoregulation. Nevertheless, renal autoregulatory response has been studied by adjustable clamp on aorta at unchanged blood volume, and the vasopressor activity is much less in such studies than in studies where the blood volume markedly reduced by bleeding. Pretreatment with indomethacin also is reported to potentiate the renal vasoconstrictor response of renal nerve stimulation, infusion of noradrenalin angiotensin (Needleman *et al.* 1976).

Our previous results are contrary to the findings in the present study where bleeding to BP 50 mmHg after treatment with indomethacin resulted in an immediate increase in renal vascular resistance, probably explained by an augmented vasopressor activity in this group.

Since the prominent effect of indomethacin is to block PGE synthesis, it is indicated that the observed vasodilation in the first phase of HH was induced by PGE. This is supported by the finding of increased concentration of PGE in arterial blood during HH reported by

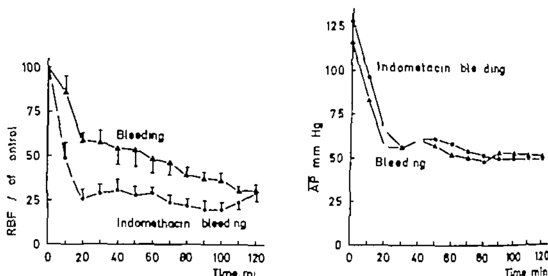


Fig. 2. Average total renal blood flow (RBF) (±S.E.) and arterial pressure ($\overline{AP}$) during bleeding (n 7) (triangles) and during bleeding after pretreatment with indomethacin (n = 9) (circles).

Generally in the indomethacin treated dogs RBF in the early bleeding phase decreased abruptly to 25% of initial flow and thereafter was practically unchanged for the rest of the expt. (Fig. 2) In the untreated group however RBF fell only to 60% of control during the first 20 min, but thereafter showed a gradual decrease of flow for 1.5 h In both groups average $\overline{AP}$ fell to the same degree during the first 20 min and ended at the same pressure within 30 min, thus renal vascular resistance was markedly higher in the indomethacin treated group After about 2 h of hypotension relative RBF in the two groups were almost identical and about 30% of initial control Similar differences in flow reduction in the early phase of bleeding, of indomethacin treated and untreated dogs, were found also by the local blood flow measurements in outer and inner cortex The volume bled from the indomethacin treated dogs was in order of 0 7 l, which was distinctly lower than the untreated group (1 2 l) suggesting an improved compensation of the blood volume loss in the last group.

The vascular conductance of outer and inner cortex (OCF/$\overline{AP}$ and ICF/$\overline{AP}$) of the indomethacin treated dogs showed an immediate great reduction during the first 15 min of bleeding (Fig. 3) A short lasting increase in conductance occured as the bleeding velocity was decreased at $\overline{AP}$ about 50 mmHg (In 1 dog ICF/$\overline{AP}$ for some minutes rose even above initial control) After about 1 h average vascular conductance in outer and inner cortex was reduced to a level of approximately 60–70% of prebleeding value.

The untreated dogs showed opposite conductance changes for the first period of HH as the conductance for the whole kidney and different cortical layers increased in all but 1 animal The average conductance was above control for 40–50 min before the conductance decreased to the same level as in the indomethacin treated dogs.

Discussion

Intravenous injection of indomethacin under control conditions changed neither total renal blood flow nor cortical flow distribution by more than a few per cent. Kirschenbaum *et al* (1974) and Swain *et al* (1975) on the other hand reported 45 and 25 decrease of

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several authors (Flynn, Appert and Howard 1975 and Johnston and Selkurt 1976). As vascular resistance and renal blood flow after 2 h bleeding was similar in the present 2 series of expts. the vasodilation caused by PGE release must be temporary or its effect suppressed by increased vasoconstrictor influence also in the untreated group

Renal blood flow distribution Carriere *et al* (1966), Truninger *et al* (1966) and other using [85]Kr and [133]Xe washout technique, reported a reduced blood flow in outer cortex during HH while blood flow in juxtamedullary cortex and outer medulla was well preserved. These results have been supported by Slotkoff *et al* (1971) and Rector *et al.* (1972) showing an increased fraction of microspheres to inner cortical nephrons in hypotensive state. Data *et al* (1976) demonstrated a pronounced reduction in blood flow to inner cortex as well during HH after pretreatment with indomethacin. According to Lee *et al* (1967) renal PGE production is limited to outer medulla and Data *et al* (1976) therefore suggested that renal PGE was responsible for the selective vasodilation in inner cortex during bleeding.

On the other hand, Aukland *et al* (1973) and Kirkebø and Tyssebotn (1974) found no evidence for a redistribution of cortical blood flow during HH and Aukland and Wolgast (1968) measured a proportional reduction in total renal and outer medullary blood flow Furthermore after pretreatment with indomethacin in the present study local blood flow in outer and inner cortex showed a similar decrease during bleeding. A vasoconstrictor modulation or a vasodilatory effect of the increased arterial PGE content during bleeding, whether this is due to increased PGE synthesis outside the kidney or increased passage through the lung, probably will affect outer and inner cortical vasculature to the same degree, thus compatible with the present results

Johnston and Selkurt (1976) did not find increased renal release of PGE 1–3 h after started bleeding, but they performed no measurements in the first hour of bleeding, so the renal release may well be higher this period. Jakschik *et al* (1974) reported peak arterial PGE concentration after half an hour hemorrhage, when the discrepancy between the two groups are greatest in our study

If equal changes of blood flow in both cortical zones anyhow depends on endogenous renal release of PGE, this is compatible with recent data (Pong and Levine 1976, Olsen and Ahnfelt Rønne 1976), demonstrating PGE synthesis both in outer and inner cortex in dog. It should be remembered that the hydrogen washout method allow repeated measurements of local peritubular blood flow whereas the microsphere method measure glomerular blood flow

In conclusion Pretreatment with indomethacin inhibit renal vasodilation in the early period of bleeding both in outer and inner cortex, indicating a temporary effect of PGE in all parts of cortex under hypotension.

While it is well known that the concentration of several vasoconstrictors in arterial blood increases radically during HH the present results suggest that their effect on the kidney is effectively but transiently counteracted by PGE in all parts of the cortex.

This work was supported by the Norwegian Council of Science and the Humanities. — Thanks are due to Professor K. Aukland for encouragement and helpful advices. Gratitude is also expressed to our cooperative technical assistants, Å Rye Eriksen and S. Lepsøe and to A/S Dumex kindly supplying indomethacin.

The nerves were stimulated with rectangular pulses of 2 ms duration with an intensity 5 times greater than that initially required for vigorous muscle twitching (supramaximal stimulation). The preparations were stimulated at 1, 2, 5 and 10 Hz for 10 min at room temperature (21–23°C).

Assay of HMTLS after stimulation of the phrenic nerve-diaphragm preparation via the phrenic nerve. At the end of the stimulation period, the tissue preparations were frozen with liquid nitrogen and then homogenized in 5 ml allylcyanide containing 12.5 mg/ml sodium-tetraphenylboron, extracted, chromatographed and measured as described in a previous paper (Waldenlind, in press). Measurements were also made of the perfusion fluid only (5). In 5 expts. the non-innervated parts of the diaphragm muscle were immediately cut off after the stimulation as described by Potter (1970) hereafter MTLS was assayed as above.

Effect of thiamine on muscular contractions and on the formation of MTLS. The effect of different thiamine concentrations (10^{-4}, 5 · 10^{-4}, 10^{-3}, 5 · 10^{-3} M) on the muscular contractions were studied when the phrenic nerve was stimulated at a frequency of 1 Hz. The muscular contractions were registered on a Grass Polygraph. A possible dose-dependent relationship between impulse-frequency (1 and 5 Hz) and the formation of MTLS was investigated in the presence of thiamine at a dose (5 · 10^{-3} M) which blocked the muscular contractions.

Effect of d-tubocurarine on the formation of MTLS. The effect of d-tubocurarine in different doses of (2, 6 and 1 mg/l) on the formation of MTLS in the phrenic nerve-diaphragm preparation was investigated. Stimulation was carried out at 5 Hz.

Release of ^{35}S-thiamine into the baths after stimulation of the phrenic nerve. 200 µCi of ^{35}S-thiamine was given to the rats 24 h before the expts. The phrenic nerve-diaphragm preparation was dissected as described above. It was necessary to keep the muscle preparations in 1 litre of bath medium for 3 h to minimize the efflux of radioactive thiamine from the muscle. Stimulation (1, 2 and 5 Hz for 10 min) was carried out in the presence of d-tubocurarine (12 mg/l). This dose is twice that required to inhibit muscle contraction due to phrenic nerve stimulation. The stimulation was followed by a rest period of 10 min. Thereafter the procedure was repeated twice. The bath medium was changed after each stimulation and rest period. The bath medium (5 ml) was added to 10 ml of Instagel® and the ^{35}S-radioactivity was counted on liquid scintillation spectrometer. In 4 expts. the radioactivity of the baths was analyzed for identity with thiamine using ion pair extraction and paper chromatography as described earlier (Waldenlind, in press).

Formation of MTLS in chopped denervated diaphragms. The left hemidiaphragm was denervated under ether anesthesia by cutting the left phrenic nerve through an incision in the anterior chest wall. After 21 days, the right and left diaphragms were taken out, chopped finely with scissors and placed in 2 ml of Tyrode solution containing 40 µCi of ^{3}H-ACh (0.16 µmol) or 40 µCi of ^{3}H-ACh (0.16 µmol) or 40 µCi of ^{3}H-choline (0.16 µmol) and thiamine (10^{-4} M final conc.). After 3 min the tissue was extracted and chromatographed as described above. The number of expts. is shown in Table I. The radioactivity was calculated as the percentage of the total added radioactivity per gram wet weight of tissue (an innervated hemidiaphragm weighed about 1.5 g and denervated about 0.5 g).

Inhibition of nicotine-induced contractions of the guinea-pig ileum by thiamine. Segments of terminal guinea-pig ileum were suspended in oxygenated Tyrode solution. The muscle contractions were registered on a smoked drum. The effects of nicotine (ED_{50}) and ACh (ED_{50}) on the contraction of the isolated ileum were compared in the presence and absence of hexamethonium and thiamine. The effect of thiamine (300 µM) on adrenaline, histamine and serotonin-induced contractions or relaxation was also measured. Adrenaline, histamine and serotonin were used in concentrations that gave a response which was 50% of the maximal one. Thiamine was added 1 min before the substance to be tested. Every substance was tested at least 5 times. The number of experiments is shown in Fig. 3.

Addition of synthetic C2-methyl thiamine to homogenates of diaphragms. 2-methylthiamine was synthetized as described earlier (Waldenlind, in press). 0.25 µmol of 2-methylthiamine was added to homogenates of diaphragms (0.69 g wet weight) in 5 ml of bath medium. 20 min later the mixture was extracted as described above (n 6). Methylthiamine was assayed by thin layer chromatography as described earlier (Waldenlind, in press). The spots were developed by Dragendorff reagent.

Materials

Choline chloride, (N-($C^{3}H_{3}$)$_3$) (1 Ci/mmol); acetylcholine chloride, (N-($C^{3}H_{3}$)$_3$) (250 mCi/mmol) and ^{35}S-thiamine hydrochloride (150 mCi/mmol) were obtained from the Radiochemical Centre, Amersham, England. Thiamine hydrochloride, nicotine, hexamethonium bromide, eserine sulphate and d-tubocurarine chloride were obtained from Sigma Chemical Company. Other chemicals were of the purest grade possible and obtained from usual commercial sources.

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From the Department of Pharmacology Karolinska Institutet, Stockholm Sweden

Release of Thiamine and Formation of a Methylthiamine-Like Substance in the Phrenic Nerve-Diaphragm Preparation of the Rat

By

LENNART WALDENLIND

Received 3 February 1977

Abstract

WALDENLIND L, *Release of thiamine and formation of a methylthiamine-like substance in the phrenic nerve-diaphragm preparation of the rat* Acta physiol scand. 1977 101 22 27

An increased formation of a radioactive methylthiamine like substance (MTLS) in the end plate region of the rat diaphragm muscle and a release of radioactivity chromatographed as thiamine into the bath medium after a subcutaneous injection of ^{35}S-thiamine were found after stimulation of the phrenic nerve. There was also an increased formation of the radioactive methylthiamine-like substance in the denervated diaphragm preparation incubated with radioactive acetylcholine (ACh) in comparison with the innervated diaphragm

The formation of a methylthiamine-like substance (MTLS) in nerve tissue has recently been described (Waldenlind, in press) In the previous publication MTLS was designed "compound A The MTLS was characterized by ion pair extraction and subsequent chromatography after labelling the nervous tissue with H-choline or ^{35}S-thiamine. It was found that the formation of radioactive MTLS was correlated with the formation of radioactive ACh and not with phospholipid formation or with the intermediary metabolism of choline.

To investigate whether or not MTLS was formed during cholinergic nerve stimulation—either in the nerve or in the innervated organ—the stimulated and denervated phrenic nerve-diaphragm preparation was used in the present study In addition, the release of radioactivity chromatographed as thiamine into the bath fluid was measured One possibility is that MTLS may in part be formed postsynaptically from released thiamine.

The action of thiamine on the function of different transmitter receptors was studied on the guinea-pig ileum preparation

Experimental

Animals. Male Sprague-Dawley rats (100-150 g) were used I some expts. the distal part of the guinea-pig ileum preparation was used.

Muscle preparations The phrenic nerve-diaphragm preparations were set up accordi g to Potter (1970) 1 μM of thiamine-hydrochloride and 1 μC of H-choline (30 μM final conc.) were added to the bath fluid

The nerves were stimulated with rectangular pulses of 2 ms duration with an intensity 5 times greater than that usually required for vigorous muscle twitching (supramaximal stimulation). The preparations were stimulated at 1, 2, 5 and 10 Hz for 10 min at room temperature (21–23°C).

Assay of H-MTLS after stimulation of the phrenic nerve-diaphragm preparation via the phrenic nerve. At the end of the stimulation period, the tissue preparations were frozen with liquid nitrogen and then homogenized in 5 ml allylcyanide containing 12.5 mg/ml sodium-tetraphenylboron, extracted, chromatographed and measured as described in a previous paper (Waldenlind, in press). Measurements were also made of the perfusion fluid only (5). In 5 expts. the non-innervated parts of the diaphragm muscle were immediately cut off after the stimulation as described by Potter (1970) hereafter MTLS was analyzed as above.

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Effect of d-tubocurarine on the formation of MTLS. The effect of d-tubocurarine in different doses of (2, 6 and 12 mg/l) on the formation of MTLS in the phrenic nerve-diaphragm preparation was investigated. Stimulation was carried out at 5 Hz.

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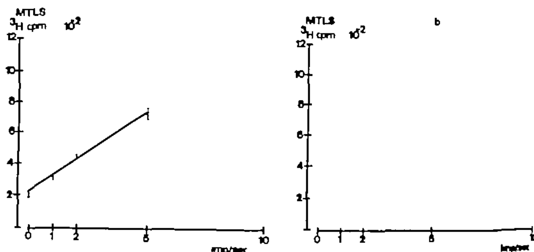


Fig. 1 Formation of MTLS at the phrenic nerve-diaphragm preparation after stimulation of the phrenic nerve. Stimulation was carried out in the presence of unchanged bath medium (a) in the presence of 10^{-4} M thiamine (b) and in the presence f d-tubocurarine (c). The regression lines in (a) and (c) were constructed using the method of least squares. The slopes of the curves were significantly different from zero In (b) the slope of the calculated regression line was not statistically different from zero a) MTLS (cpm) in unstim lated and stimulated preparations (1 2 and 5 Hz). The stimulatio at 10 H was not included n the calculation of the regression line. 10 expts. were carried out at each stimulation frequency b) MTLS after stim lation i the presence f a high dose of thiamine. 8 or 9 expts. were carried ut at each stim lation frequency c) MTLS after stimulation (5 H) i the presence of different doses of d-tubocurarine. 10 expts. were carried out t each concentration.

Results

Increase of H MTLS in the end-plate regions after stimulation of the phrenic nerve-diaphragm preparation. When the phrenic nerve was stimulated there was a stimulus-dependent increase of extractable H MTLS from the diaphragms up to 5 Hz (Fig. 1 a). No measurable MTLS was formed in the muscle outside the end-plate region No MTLS could be extracted from the bath medium (n = 5).

Effect of thiamine on muscular contractions and on the formation of MTLS Thiamine at a concentration of 1–5 10^{-4} M inhibited the contraction of the diaphragm The inhibition was dose-dependent. When the muscle was paralysed by thiamine (10^{-4} M) no frequency dependent formation of MTLS was observed (Fig. 1 b)

Effect of d-tubocurarine d-Tubocurarine inhibited the formation of the MTLS in a dose-dependent matter (Fig. 1 c)

Release of ^{35}S-thiamine into the baths after stimulation of the phrenic nerve A stimulus-dependent release of radioactivity chromatographed as thiamine into the bath medium was

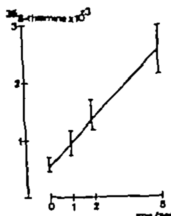


Fig. 2. Release of ³⁵S-thiamine into the bath medium when the phrenic nerve-diaphragm preparation was stimulated via the phrenic nerve in the presence of d-tubocurarine (1 mg/l). The relation between stimulation frequencies and the release of ³⁵S-thiamine is shown by the linear regression line calculated by the method of least squares. The slope of the regression line was significantly different from zero. The standard deviation for each stimulation frequency shown in the figure (n = 4–6).

observed when the phrenic nerve was stimulated in the presence of d-tubocurarine in a dose twice as high as that which inhibited the muscle contractions (Fig. 2). In the 4 expts. where the bath medium was extracted and chromatographed as before (Waldenlind, in press), 85-94% of the radioactivity was measured as thiamine. Therefore the ³⁵S-radioactivity should be a good indication of the thiamine content.

Formation of MTLS from denervated diaphragm (chopped tissue) When denervated rat diaphragms were incubated with radioactive ACh, significantly more MTLS was found in denervated than in innervated tissue (Table I). Incubation with choline alone gave no detectable formation of MTLS.

Inhibition by thiamine of the effect of nicotine on the guinea-pig ileum. The stimulatory action of nicotine (ED_{50} 10–30 μM) on the guinea-pig ileum was inhibited dose-dependently by thiamine (40–300 μM) (Fig. 3). The inhibition lasted at least 60 min. Removal of thiamine by changing the bath fluid immediately restored the sensitivity of the intestine to nicotine. Thiamine did not change the response of the intestine to ACh, histamine, serotonin or adrenaline. The effect of thiamine was found to be similar to that of hexamethonium—blockage of the response to nicotine but not to that of ACh.

Addition of C2-methylthiamine to homogenate of diaphragms No C2-methylthiamine could be extracted from the homogenate of the diaphragm muscle after the addition of synthetic C2-methylthiamine (n = 6). Only thiamine could be detected in the chromatograms.

TABLE I. Comparison of the formation of methylthiamine-like substance after incubation of chopped homogenate of innervated and denervated diaphragm in the presence of thiamine and ³H-ACh. The values are given as percentage of the totally added radioactivity per g wet weight of tissue (an innervated hemidiaphragm weighs about 1.5 g and denervated about 0.5 g). The means and standard deviations are given in the table.

	Innervated	Denervated
Amount of methylthiamine	0.079 ± 0.013 (n = 10)	0.44 ± 0.054 (n = 10)

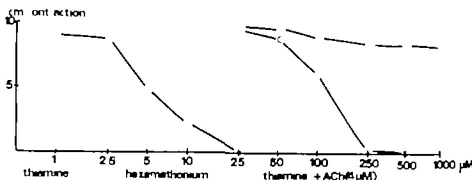


Fig. 3 Dose-dependent inhibition by hexamethonium and thiamine of nicotine-induced contraction of the intestine is shown in the two sigmoid curves. The third curve shows the effect of thiamine on ACh-induced contractions. The reponse to ACh (4 / M) showed a small decrease at thiamine concentrations between 25 and 100 μM of thiamine and was therefore not further decreased at least to a concentration of 1 mM. All curves were reproducible when tested 5 times.

Discussion

In a previous paper (Waldenlind, in press) the formation of MTLS *in vivo* especially in cat ventral roots, after a H-choline injection locally into the spinal cord has been described. The synthesis of ACh showed a time-curve similar to that of MTLS formation in contrast to the formation of phospholipids. Betaine, the oxidation product of choline which is metabolised in the intermediary metabolism by donating methyl groups, could not act as a precursor of MTLS For various reasons it was suggested that the synthesis of MTLS may reflect nicotinic receptor activity This hypothesis was to be further tested by using a physiological preparation such as the phrenic nerve-diaphragm preparation By stimulating the phrenic nerve it was thought that the cholinergic transmission was activated The production of MTLS increased as can be seen in Fig. 1 a. It was, however important to freeze the preparation immediately in liquid nitrogen—otherwise the transient increase of MTLS could not be calculated from the results The MTLS was also concentrated to the end plate region of the diaphragm muscle, which indicates a relation to nerve terminal structures. No MTLS was detected in the nerve-free parts of the muscle.

By denervating hemidiaphragms it was possible to compare the amount of MTLS formed in chopped innervated and denervated diaphragms. The amount of MTLS was about 5.6 times higher in the denervated diaphragms than in the innervated diaphragms if the calculations were based on the weight of wet tissue and about twice of the results were expressed per hemidiaphragm (the innervated diaphragms were heavier). As denervation is known to increase the number of extrajunctional ACh-receptors (Axelsson and Thesleff 1957), the increased amounts of MTLS could be related to this change Conclusions must however be drawn with care since the muscle metabolism is probably changed by the denervation procedure.

Thiamine in a dose which blocked the muscular contractions due to phrenic nerve stimulation gave no stimulus-dependent increase of MTLS (Fig. 1 b) Instead the basal levels of MTLS were elevated at all stimulation frequencies suggesting that the formation of MTLS was stimulated In contrast d tubocurarine inhibited the formation of MTLS in a dose-

dependent manner (Fig. 1 c). This was the same dose-range as that which blocked the muscular contractions.

In addition, a release of ³⁵S-thiamine was found from stimulated curarized phrenic nerve diaphragm preparations. An attractive idea is that the MTLS was formed from released ³⁵S-thiamine. This suggestion is, at least on the basis of the present expts., only a speculation. An increased release of thiamine from the phrenic nerve has been described by Minz (1938). A microbial method for thiamine assay was used.

Thiamine inhibits the diaphragm contractions elicited by phrenic nerve stimulated and the nicotine-induced response of the guinea-pig ileum (in 50–100 μM concentration). The effect on the guinea-pig ileum was a specific inhibition of the nicotine-induced contractions. The effect of other amines tried were not changed. Thus it seems that thiamine has a specific affinity to structures mediating the nicotine response. Similar results have been described by Unna and Pick (1944).

The results reported in the present paper support the hypothesis that activation of nicotine receptors leads to formation of MTLS. It is, however possible that MTLS is related by another mechanism to nerve metabolism. The expts. with the phrenic nerve-diaphragm preparation do not show whether the synthesis of MTLS occurs presynaptically or postsynaptically. The "cholinoreceptors" in the denervated diaphragm are not identical with the postsynaptic ones and, besides, reinnervation might have occurred to some extent.

In order to try to rule out the possibility that MTLS is an artefact of the procedure used, blank extractions and chromatograms with radioactive choline and ACh were made as had been described in a previous paper (Waldenlind, in press). MTLS could only be found if choline was allowed to be metabolised in an unhomogenized tissue. Ion-pair extraction from homogenized tissue to which choline or ACh had been added yielded no MTLS. Despite these controls, external factors influencing the extraction or chromatography and leading to an artefact described as MTLS may have been overlooked, although this must be considered improbable.

A role for thiamine in neuroconduction has been proposed by v. Murali (1962) and Cooper (1968). They found an increased release of thiamine from stimulated nerves. In future investigations, the importance of thiamine for nicotinic receptor activity will be investigated.

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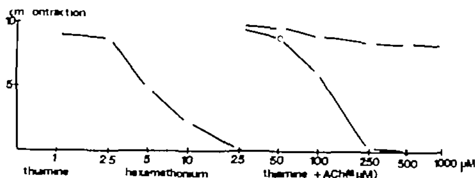


Fig. 3 Dose-dependent inhibition by hexamethonium and thiamine of nicotine-induced contraction of the intestine is shown in the two sigmoid curves. The third curve shows the effect of thiamine on ACh-induced contractions. The reponse to ACh (4 µM) showed a small decrease at thiamine concentrations between 25 and 100 µM of thiamine and was therefore not further decreased at least to a concentration of 1 mM. All curves were reproducible when tested 5 times.

Discussion

In a previous paper (Waldenlind, in press) the formation of MTLS in vivo especially in cat ventral roots, after a H-choline injection locally into the spinal cord has been described. The synthesis of ACh showed a time-curve similar to that of MTLS formation, in contrast to the formation of phospholipids. Betaine, the oxidation product of choline which is metabolised in the intermediary metabolism by donating methyl groups, could not act as a precursor of MTLS. For various reasons it was suggested that the synthesis of MTLS may reflect nicotinic receptor activity. This hypothesis was to be further tested by using a physiological preparation such as the phrenic nerve-diaphragm preparation. By stimulating the phrenic nerve it was thought that the cholinergic transmission was activated. The production of MTLS increased as can be seen in Fig. 1 a. It was, however important to freeze the preparation immediately in liquid nitrogen—otherwise the transient increase of MTLS could not be calculated from the results. The MTLS was also concentrated to the end-plate region of the diaphragm muscle, which indicates a relation to nerve terminal structures. No MTLS was detected in the nerve free parts of the muscle.

By denervating hemidiaphragms it was possible to compare the amount of MTLS formed in chopped innervated and denervated diaphragms. The amount of MTLS was about 5.6 times higher in the denervated diaphragms than in the innervated diaphragms if the calculations were based on the weight of wet tissue and about twice of the results were expressed per hemidiaphragm (the innervated diaphragms were heavier). As denervation is known to increase the number of extrajunctional ACh-receptors (Axelsson and Thesleff 1957), the increased amounts of MTLS could be related to this change. Conclusions must however be drawn with care since the muscle metabolism is probably changed by the denervation procedure.

Thiamine in a dose which blocked the muscular contractions due to phrenic nerve stimulation gave no stimulus-dependent increase of MTLS (Fig. 1 b). Instead the basal levels of MTLS were elevated at all stimulation frequencies suggesting that the formation of MTLS was stimulated. In contrast d tubocurarine inhibited the formation of MTLS in a dose-

dependent manner (Fig. 1 c). This was the same dose-range as that which blocked the muscular contractions.

In addition, a release of ^{35}S-thiamine was found from stimulated curarized phrenic nerve diaphragm preparations. An attractive idea is that the MTLS was formed from released ^{35}S-thiamine. This suggestion is, at least on the basis of the present expts., only a speculation. An increased release of thiamine from the phrenic nerve has been described by Minz (1938). A microbial method for thiamine assay was used.

Thiamine inhibits the diaphragm contractions elicited by phrenic nerve stimulated and the nicotine-induced response of the guinea-pig ileum (in 50–100 μM concentration). The effect on the guinea-pig ileum was a specific inhibition of the nicotine-induced contractions. The effect of other amines tried were not changed. Thus it seems that thiamine has a specific affinity to structures mediating the nicotine response. Similar results have been described by Unna and Pick (1944)

The results reported in the present paper support the hypothesis that activation of nicotine receptors leads to formation of MTLS. It is, however possible that MTLS is related by another mechanism to nerve metabolism. The expts. with the phrenic nerve-diaphragm preparation do not show whether the synthesis of MTLS occurs presynaptically or post synaptically. The cholinoreceptors in the denervated diaphragm are not identical with the postsynaptic ones and, besides, reinnervation might have occurred to some extent.

In order to try to rule out the possibility that MTLS is an artefact of the procedure used, blank extractions and chromatograms with radioactive choline and ACh were made as had been described in a previous paper (Waldenlind, in press). MTLS could only be found if choline was allowed to be metabolised in an unhomogenized tissue. Ion-pair extraction from homogenized tissue to which choline or ACh had been added yielded no MTLS. Despite these controls, external factors influencing the extraction or chromatography and leading to an artefact described as MTLS may have been overlooked, although this must be considered improbable.

A role for thiamine in neuroconduction has been proposed by v. Muralt (1962) and Cooper (1968). They found an increased release of thiamine from stimulated nerves. In future investigations, the importance of thiamine for nicotinic receptor activity will be investigated.

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Plasma Protein Dynamics: Albumin and IgG Capillary Permeability, Extravascular Movement and Regional Blood Flow in Unanesthetized Rabbits

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Abstract

BILL, A. *Plasma protein dynamics. Albumin and IgG capillary permeability, extravascular movement and regional blood flow in unanesthetized rabbits.* Acta physiol. scand. 1977. *101*. 28–42.

The turnover rate constants for extravascular alb min and Immunoglobulin-G (IgG) and the turnover rates and extravascular spaces for the proteins and regional blood flow were determined n different tissues in unanesthetized rabbits. The t rnover rate consta t percent per minute for extravascular albumin was 1.9 in the kidney cortex and 1 4 in the kidney medulla, bout 0.8 1 4 the lung, small I testine, choroid ple us and heart muscle and about 0 2–0 6 the skeletal muscle, stomach wall nd gall bladder. The turnover rate constants for IgG were similar or lower. The plasma equivalent extravascular albumin space was 145 $\mu l/g$ the kidney medulla, bout 100 $\mu l/g$ the gall bladder heart muscle choroid plexus and small intestine, 30–70 $\mu l/g$ in the kidney cortex, lung, stomach wall and triceps muscle. The extravascular *IgG spaces were sim la r smaller. In the liver spleen nd adrenals where extravascular spaces could not* be determined, the ratios total IgG space/total albumin space were bout 1 3–1.5 E traction of lbumin def ned as turnover rate of extravascular lbumin/regional plasma flow could not be determ ned in the *liver spleen and drenals. It was zero in the bra n cortex, bo t 0 001 in the lu g and round 0 01–0 05* t most other tissues. The results suggest that in the walls of the capillaries of the kidney cortex, stomach wall, skeletal muscle a d gall bladder there was convection of the proteins with pinocytosis and/or through large pores, diffusio playing little role. The albumin and the IgG passed through the tissues and were drained essentially by bulk flow I the heart m scle, choroid plex s, lung nd small intestine there seemed to be some diffusion of album n thro gh the capillary wall n addition to con ection. Accumulation of IgG in the liver spleen and adrenals was probably caused by adsorption t cells belongi g to the RES.

Key ords: Albumin, immunoglobulin-G capillary permeabil ty tissue fl id protein, prote extraction

In most tissues there is a leakage of protein from the capillaries into the tissue fluid and a drainage of protein from the tissue by the lymph vessels. The movement of protein from the capillaries into the tissue fluid and back into the general circulation by way of the lymph vessels or other routes is of importance for the filtration of fluid from the capillaries. It is also of importance for many other mechanisms—such as the defence against antigenic

substances, the replacement of vascular proteins after blood loss, and the distribution of fatty acids, many hormones, vitamins and drugs.

Studies on lymph flow and lymph composition have contributed greatly to the understanding of several important aspects of the extravascular exchange of macromolecules in anesthetized animals (Grotte 1956, Garlick and Renkin 1970, Johnson and Richardson 1974) and in heart-lung preparations (Arturson, Groth and Grotte 1972).

Grotte's (1956) studies on the movement of dextrans of different molecular weights from plasma to lymph indicated that there are two kinds of functional pores in the walls of the capillaries: small pores with a diameter of about 8 nm and large pores or leaks with diameters of 24–70 nm (see also Pappenheimer 1970). The plasma proteins were thought to pass out through the large pores. The results of later estimates of pore sizes have varied somewhat but the two pore-size hypothesis has gained increasing support (Michel 1972, Crone 1974).

In some tissues, e.g. liver and spleen, the large pores are easily identified, there are gaps in the capillary walls which are even larger than 70 nm (Majno 1966). In other tissues the conditions are less clear. In the capillaries of skeletal, heart and smooth muscle, no clearcut candidates for the role as real large pores have been found. This may be due to a very low frequency of large pores or actual pores may be absent. In such tissues pinocytotic vesicles, which have been observed to transport molecules as large as ferritin with a diameter of 11 nm may very well be the only representative for the large pore system (Karnovsky 1962, Renkin 1964, Wanne 1965). Strong evidence for this hypothesis was presented by Garlick and Renkin (1970). In other organs, e.g. the small intestine, parts of the endothelial cells of the capillaries are very thin and in these places there are large numbers of fenestrations. These fenestrations have a diameter of about 40 nm nicely within the limits for the large pores predicted by Grotte. However in electron micrographs these fenestrations are often closed by a thin membrane. It is not clear if this membrane is present in all fenestrations or just a proportion under in vivo conditions, and it is possible in fact that there are differences between organs in this respect. Studies with marker molecules have indicated that macromolecules may pass through fenestrae as well as with pinocytosis (Simionescu, Simionescu and Palade 1972).

Elaborate classifications of capillaries take into account the ultrastructure of the endothelial cells, the basement membrane and the pericapillary investment (Bennett, Luft and Hampton 1959). But in this discussion capillaries will be referred to as gap capillaries and as fenestrated and continuous capillaries. Protein exchange taking place in venules and small veins will be included with that taking place through the capillary walls.

Lymph studies of course can give information only about the net outflow of macromolecules from the capillaries. A usual assumption was that there is little return of macromolecules into the capillaries within the tissues. This may be true for some tissues but return of protein into the general circulation within the tissue has been reported for the liver (Goresky 1970), the kidney (Swann 1960), and skeletal muscle (Perry and Garlick 1975). It has been argued (Casley-Smith, O'Donoghue and Crocker 1975) that the reabsorption of protein into the venous part of the capillaries may be a usual phenomenon in tissues with fenestrated capillaries.

Studies on the accumulation of labelled albumin in the various tissues of mice have been

reported by Friedman (1957) and Dewey (1959) Studer and Potchen (1971) have reported data for many tissues in rats and Owen and Triffitt (1976) have presented data for some tissues in rabbits. Such data are of great interest because they can be used to calculate the volumes of the extravascular albumin spaces and the rate of outflux of albumin from the capillaries. But they give no information about the mechanism for protein drainage from the tissues.

Previous communications (Bill 1968 a, b) reported on the accumulation of labelled albumin and gammaglobulin in some tissues by employing a double isotope method. These studies, centered on intraocular tissues, showed that simultaneously determined spaces may enable a more detailed analysis of the extravascular movement of proteins than studies on single proteins.

The purpose of this investigation was to extend the previous study to other tissues. The regional blood flow was determined in order to relate protein outflux from the capillaries to the plasma flow

Methods

Albin rabbits of both sexes, weighing 2 3 kg each, were used in the determinations of the protein spaces and the regional blood flow The animals were given pellets and water ad lib. before and during the expts and were kept t a room temperature of bout 22°C. Each animal was free to move in a cage ith a floor space of about 40 50 cm. Rabbits weighing 0 8–1.0 kg were used in the screening of the proteins.

1 *Experiments with ^{131}I-albumin and ^{125}I-albumin*

The animals were given albumin labelled with one iodine isotope n such a way that the concentration in the plasma was kept almost constant for a period of time varying from 15 min to 48 h (Bill 1968). Albumin labelled with the ther iodine isotope was given for shorter period of time n such way that the plasma concentration f this albumin was also kept almost constant until the end of the expts. The animal s killed by a rapid injection of 60 mg/kg b wt. pentobarbital sodi m followed by 3 ml saturated KCl

2. *Experiment with ^{131} I-albumin and ^{125}I-immunoglobulin-G*

The labelled proteins were njected i. Additional injections kept the plasma concentration of the two proteins almost consta t.

I these expts. screened proteins were used except a few preliminary expts. The t tal injected olumes were less than 10 ml.

Labelled proteins Human serum albumin labelled with ^{131}I or ^{125}I was obtained from AB Atomenergi, Studsvik. Rabbit serum lbumin and IgG were purified by gel chromatography on Sephadex G 200 (Pharmacia, Uppsala) nd labelled with ^{125}I or ^{131}I ccordi g to McFarlane. T remove iodine not bound to the protein, the proteins were subjected t gel chromatography on Sephadex G 25 mmediately before use. The results obtained with the different labelled albumins indicated that there was a small fraction of the albumin that was eliminated very quickly from the blood stream by the kidneys The proteins were screened i order to remove this probably slightly denaturated protein. Fo screening the proteins were njected i int small rabbits. The plasma obtained from these rabbits 30–60 min later was given t the animals in which the turnover rates were to be determined.

Assay The radioactivity was determined by means f two-cha nel gammaspectrometry Precipitation with 20 % trichloroacetic acid was used t determine the amount of iodine not bount to the protei in the blood and tissue samples (for details see Bill 1968). Plasma equivalent albumi and IgG spaces were calculated as in the previous tudy

3 *Regional blood flow*

The regional blood flow was determined in unanesthetized rabbits ne day after the cannulation of the left heart ventricle for microsphere injection nd one femoral artery for the collection of reference sample.

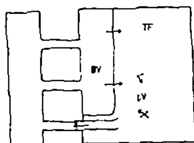


Fig 1 Model 1 for extravascular exchange of proteins discussed under "Theory". BV represents the blood vessels of the tissue; TF- tissue fluid, LV the lymph vessels. Arrows indicate direction of protein fluxes.

The heart was cannulated via the left brachial artery. The spheres were 15 μm carbonized microspheres (3M Company), labelled with ⁸⁵Sr. The regional blood flow in g min⁻¹ g⁻¹ was calculated as described elsewhere (Bjerneskantz, Alm and Bill 1976). The plasma flow in ml min g⁻¹ was calculated as blood flow × (100 − haematocrit)/(100 × 1.05)

Theory

The results of the previous study (Bill 1968) and those of the present expts indicate that extravascular plasma protein exchange varies from one tissue to another. A rather simple model seems to describe the situation in some tissues while in others the results are somewhat ambiguous.

Fig 1 shows the simple model 1. The pathways between BV and TF may be pinocytotic vesicles operating in one direction only or actual pores in which outward filtration is so rapid that return of the macromolecules by diffusion is hindered (see Bill 1968). The membrane separating TF from LV is assumed not to discriminate between the albumin and IgG which enter LV by the bulk flow of the tissue fluid. In this model LV may then be included in TF.

If radioactive albumin is added to the general circulation and the plasma concentration is kept constant, compartment BV will reach a steady state concentration of the labelled albumin within a few min. Albumin will also enter TF. Diffusion of albumin in the tissue and the movement of the tissue fluid will give an apparent "mixing" of the tissue fluid. Labelled albumin is therefore also lost from the TF by way of the lymph vessels almost from the start. After some time a steady state is reached. The specific activity of the albumin in the tissue fluid then is the same as that in the plasma. The replacement of the unlabelled albumin originally present in the TF by albumin having the same specific activity as the plasma is described by the equation:

$$EAV = ES_{max}\, e^{-k_{alb} t}$$

where EAV is the apparent volume per g tissue of unlabelled albumin left in the tissue at time t, and ES_{max} is the plasma equivalent extravascular albumin space per g tissue—defined as mg alb/g tissue divided by mg alb/ml plasma. This volume is equal to the steady state extravascular plasma equivalent albumin space measured with radioactive albumin, S_{max}. The k_{alb} is the turnover rate constant for albumin in TF. EAV at all times equals $S_{max} - S_{t}$, where S_{max} is the steady state plasma equivalent albumin space per g tissue and S_{t} the apparent plasma equivalent albumin space at time t, as determined with labelled albumin.

$$EAV = S_{max} - S_{t} = S_{max}\, e^{-k_{alb} t}$$

In a semilogarithmic plot of $S_{max} - S_{t}$ versus time a line of best fit may be drawn, and the T1/2 can be read and the turnover rate constant for albumin, k_{alb}, can be calculated: $k_{alb} = 0.693/(T1/2)$. The S_{max} equals the intercept of the line with the ordinate. The plasma volume PBV remaining in BV after the death of the animal and the dissection can be calculated as

$$PBV = S_{tot} - S_{max}$$

See Bill (1968) and Fig 4

The same considerations as above can be made for IgG having the turnover rate constant k_{IgG} in TF. On the assumptions made, k_{alb} and k_{IgG} should be very similar in the same tissue. This is true since the turnover rate constants will differ only if there is a barrier in the route draining the proteins that restricts

the movement of the IgG more than that of the albumin or if diffusion plays a great role in the elimination of the proteins.

The sieving in the capillary walls makes the steady state albumin space in TF larger than the corresponding steady state IgG space—as a consequence the turnover rate of albumin in TF defined as k_{ab} S, will be larger than the IgG turnover rate determined analogously

Since model 1 assumed no return of protein into the blood vessels, the turnover rate for albumin equals the apparent leakage of the plasma equivalent fluid (with respect to albumin) out of the capillaries. It is equal to the drainage of such fluid from the tissue by the lymph vessels. The turnover rate for IgG analogously equals the apparent leakage of the plasma equivalent (with respect to IgG) fluid out of the capillaries and its drainage by the lymph vessels. The ratio turnover rate for IgG/ the turnover rate for albumin gives a measure of the restriction to outflow of IgG as compared to albumin in the capillary wall. There is one source of ambiguity a return of plasma proteins into the capillaries without discrimination between albumin and IgG cannot be distinguished from bulk drainage into the lymph vessels.

Model 2 which covers several alternatives differs from model 1 in that the extravascular IgG has a lower turnover rate constant than the albumin. Some mechanisms that may give a real or an apparent drainage hinderance for the IgG are a) elimination of protein from the tissue by diffusion back into the capillaries through pores or movement back into the capillaries with size discriminating pinocytosis, b) flow of tissue fluid through a barrier in the tissue or the lymph capillary wall that is more permeable to the albumin than to the IgG c) adsorption of the IgG to some tissue component and d) movement by diffusion over relatively large distances that will make IgG lag due to its lower diffusion coefficient.

The turnover rate for albumin again gives the apparent leakage of albumin out of the capillaries but the net outflow may be less because of the albumin returning into the capillaries. The same reasoning holds for the IgG The ratio turnover rate for IgG/turnover rate for albumin gives a measure of the relative restriction in the capillary wall to IgG compared to that of the albumin.

It would ideally be possible to decide if model 2 behaviour is due to a return of the albumin by diffusion and/or pinocytosis into the capillaries (Model 2 A) or to some mechanism resulting in marked filter effect (Model 2 B) In the former case one can expect the turnover rate of albumin to be much faster than that of IgG In the latter case one can expect a normal relationship between the turnover rates but a large IgG space as compared to the albumin space

Results

Albumin spaces

In the expts. with I-albumin and ¹³¹I-albumin steady concentrations in the plasma of the two labelled proteins were maintained for two different periods of time. As a consequence 2 values were obtained for the plasma equivalent albumin spaces in each sample In the expts with albumin and IgG only one value per sample was obtained for the plasma equivalent albumin space. The values determined for the albumin space each time were pooled for each tissue. The mean values, usually calculated from 4 samples of each tissue in each of 10–2 rabbits, were plotted against time, Fig. 2 A

In several expts with unscreened human and rabbit serum albumin it was found that the kidney cortex had a larger 15 min albumin space than the 6 h space determined in the same animal This result was seen irrespective of the albumin used to determine the 15 min space This indicated that both ¹²⁵I-albumin and ¹³¹I-albumin contained a small fraction of denaturated labelled protein that was rapidly eliminated by the kidneys. In order to eliminate artifacts caused by such denaturated albumin, S at different times was determined with screened albumin in the kidney and some of the other tissues. In this series of experiments the 5 and 30 min space was determined in five animals and the difference was calculated for each tissue. Other space pairs determined were the 30 and 60 min spaces, the 1 and 4 h spaces and the 4 and 8 h spaces. The 4 h values and the differences observed were used to construct S_{at} versus time plots (Fig. 2 B). Usually 4 samples were taken from each tissue in



Fig. 2 A. The apparent plasma equivalent albumin space in lung, skin and skeletal muscle at different times after the injection of labeled albumin. Mean values and standard errors are shown.

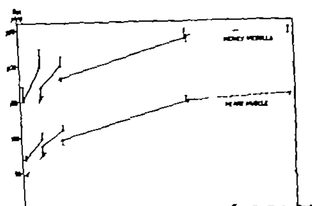


Fig. 2 B. Observed values for the plasma equivalent albumin spaces at different times—solid symbols, and values calculated from the 4 hour spaces and the differences observed—open symbols.

5 animals. With screened albumin the $S_{a,t}$ increased with time—as expected—in the kidneys. For the other tissues investigated, the results obtained with screened albumin were similar to those obtained with the unscreened. In semilogarithmic plots of $S_{\infty} - S_{a,t}$ versus time there was a much better fit between the line and the points with the second method than with the first. The reason for this was no doubt that variations in the blood volume between animals did not affect the results of the second method.

Table I shows the values for the rapidly exchanging plasma equivalent spaces which were assumed to be intravascular and the volumes of the extravascular spaces calculated from the semilogarithmic plots, the turnover rate constants for albumin, and the turnover rate for albumin in the tissues. The values shown were obtained both with unscreened and screened albumin for the skin, the triceps muscle and the lung. For the other tissues the values were calculated from the expts. with screened albumin only. In the brain, liver, spleen, bone marrow and adrenals the increase with time in the calculated albumin space from 5 min and onward was less than 10 %. No meaningful values could be calculated for the turnover rates and the extravascular spaces in these tissues.

For the skin no turnover rate constant could be determined. From the appearance of the

TABLE I Data for albumin spaces

Tissue	Fast albumin space µl g⁻	Slow albumin space, $S_{a,ss}$ µl g⁻	$k_{a,ts}$ min⁻¹	$k_{a,ts}$ $S_{a,ss}$ µl min⁻¹ g
Kidney medulla	90	145	0 014	2.03
Heart muscle (left ventricle wall)	44	115	0.011	1.26
Kidney cortex	59	64	0.019	1.20
Small intestine	21	84	0 013	1.09
Choroid plexus	137	93	0.0096	0.89
Gall bladder	47	107	0.0060	0 64
Lung	124	72	0.0083	0 58
Stomach wall (corpus)	20	58	0.0030	0 17
Skin	11	—	—	0 066
Triceps muscle	4	30	0.0017	0 051

plot for the apparent albumin space versus time, it seemed reasonable to assume tha the 2 hours following the first injection of labelled albumin, only a negligible elimin extravascular labelled protein occurred in this tissue—the space increased almost An approximative value for the turnover rate of the albumin could then be calcu dividing the difference between the 2 h and 5 min spaces by the variance in time.

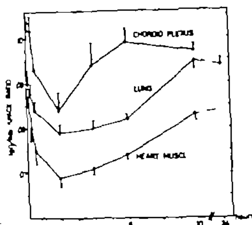
Albumin and IgG spaces

In these expts. the plasma concentration of the two labelled proteins was kept alm stant from the beginning of the expts. until the animal was killed. The apparent equivalent albumin and IgG spaces, S_{at} and S_{gt}, respectively were calculated at d times. For most tissues there was such variability in the spaces that the turnover ra stants and turnover rates could not be determined with accuracy from the space data In each expt for each tissue, S_{gt} was divided by S_{at} to obtain the IgG/albumin spac SR. The value for the space ratio each time varied moderately for each tissue. Fig. plots of IgG/albumin space ratios versus time.

The space ratios were used to construct curves describing the accumulation in the of labelled IgG For this purpose values for the idealized S_a denoted S_{at} were cal from the line in the semilogarithmic plots of $S_{ass} - S_{at}$ versus time. To obtain S_{IgGt} alized value was multiplied by the pertinent SR denoted SR

$$S_{IgGt} = SR \quad S_{at}$$

For each tissue $S_{IgGss} - S_{IgGt}$ was plotted against time and the turnover rate consta vascular space and the apparent extravascular space were calculated as for the all Fig. 4 shows the semilogarithmic plot for heart muscle. Table II III and IV show the r Space ratios at 5 and 30 min and 24 h were based on determinations of the means of 4 from 5 animals (n = 5) Each of the ratios at 15 min 2, 4 6 and 10 h was based on e ments in 12 animals. The whole choroid plexus was taken as one sample



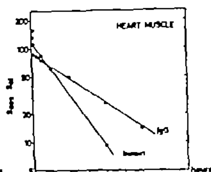


Fig. 3

Fig. 4

Fig 3 The IgG/albumin space ratio for different tissues was plotted against time. Mean values and standard errors are shown

Fig 4 A semilogarithmic plot of the steady state albumin space minus the space at different times versus time. The line was drawn by eye and T 1/2 determined from the line. The intercept of the line with the space axis gave the apparent volume of the extravascular space. The difference between the intercept and the steady state also—circle—was the volume of the intravascular space in dead animals. The idealized value for the albumin space at different times was determined using the line. This value multiplied by the pertinent space ratio gave the apparent volume of the IgG space at different times. The steady state plasma equivalent IgG space—open square—minus the space at different times was also plotted versus time and the T 1/2 and apparent volumes of extravascular and intravascular spaces were determined as for the albumin.

Monoexponential curves seemed to describe the situation in all tissues both for the albumin and the IgG

Regional blood flow protein extraction

Table V shows the values for the regional blood flow (n = 10) and albumin- and IgG-extractions. Extractions were calculated as turnover rate/regional plasma flow and expressed as percentages.

TABLE II Data for IgG spaces

Tissue	Fast IgG space $\mu l\ g^{-1}$	Slow IgG space, S_{slow} $\mu l\ g^{-1}$	k_{IgG} min^{-1}	$k_{IgG}\ S_{slow}$ $\mu l\ min^{-1}\ g^{-1}$
Kidney medulla	82	116	0 011	1.27
Heart muscle (left ventricle all)	43	88	0 005	0 44
Kidney cortex	54	56	0 019	1.06
Small intestine	21	78	0 008	0 60
Choroid plexus	137	81	0 0040	0 36
Gall bladder	46	86	0 0060	0 52
Lung	134	68	0 0029	0 1
Stomach wall (corpus)	20	36	0 0030	0.11
Skin	11	—	—	0 050
Triceps muscle	4	15	0 0017	0 025

TABLE III Ratios and capillary ultrastructure

Tissue	Turnover rate ratio $\frac{k_{IgG}}{k_{alb}}\frac{S_{eIgGss}}{S_{ealbss}}$	Turnover rate constant ratio $\frac{k_{IgG}}{k_{alb}}$	Extravascular space ratio $\frac{S_{eIgGss}}{S_{ealbss}}$	Ultrastructure of capillaries
Kidney medulla	0.63	0.8	0.80	Fenestrated
Heart muscle (left ventricle wall)	0.35	0.5	0.77	Continuous
Kidney cortex	0.88	1.0	0.88	Fenestrated
Small intestine	0.55	0.6	0.93	Mainly fenestrated
Choroid plexus	0.40	0.4	0.87	Fenestrated
Gall bladder	0.81	1.0	0.80	Mainly fenestrated
Lung	0.36	0.4	0.94	Continuous
Stomach wall (corpus)	0.65	1.0	0.62	Mainly continuous
Skin	0.75	—	—	Continuous
Triceps muscle	0.49	1.0	0.49	Continuous

Discussion

In tissues where the extravascular albumin spaces were larger than the extravascular IgG spaces and the turnover rate constant for IgG/the turnover rate constant for albumin was about unity model 1 describes the situation The albumin and IgG passed out of the capillaries by a process that restricted the movement of the IgG compared to that of albumin but the plasma proteins left the tissue essentially by bulk flow Bulk drainage is easily envisaged as drainage with lymph but the possibility of pinocytosis without size discrimination back into the capillaries has to be kept in mind The mechanism causing protein outflow from the capillaries is less clear There are two main alternatives, filtration through relatively large pores where diffusion plays little role (see Winne 1965 Blake and Staub 1976) and movement with pinocytosis. And it is possible, of course, that the two mechanisms might operate in parallel. Table III shows that tissues with continuous capillaries as well as tissues with fenestrated capillaries belong to this group The turnover rate of IgG/turnover rate of albumin was 0.49–0.88. Such rather variable sieving might result from convection through

TABLE IV Total plasma equivalent albumin and IgG spaces in tissues that had such rapid turnover of albumin i the tissue fluid that turnover rates co ld not be calculated. The mean alues are calculated from data t different times (= 30) V lues for bra n are also shown here. There was no indication for an extravascula protein space i the brain. IgG/albumin space ratios are also shown

Tissue	Albumin space S_{alb} µl g⁻	IgG space S_{IgG} µl g⁻	$\frac{S_{IgG}}{S_{alb}}$
Adrenals	65 ± 2	85 ± 2	1.30 ± 0.02
Liver	116 ± 4	179 ± 8	1.54 ± 0.06
Spleen	72 ± 2	98 ± 2	1.38 ± 0.02
Bone marrow (from ribs)	124 ± 5	127 ± 5	1.03 ± 0.01
Brain, cortex	5.2 ± 0.2	[illegible]	1.00 ± 0.01

TABLE V Regional blood flow, plasma flow and protein extraction

Tissue	Blood flow g min^{-1} g^{-1} M ± S E.	Plasma flow ml min^{-1} g^{-1}	Albumin extraction	IgG extraction
Kidney medulla	d.	d	d.	d.
Heart muscle (left ventricle all)	5 7 ± 0 8	3 6	0 036	0 012
Kidney cortex	6 4 ± 0 5	4 0	0 030	0 027
Small intestine	1.2 ± 0 2	0.7	0.156	0 086
Choroid plexus	3 2 ± 0 4	3 3	0 027	0 011
Gall bladder	2.2 ± 0.3	1 3	0 041	0 040
Lung	d	d.	d.	d.
Stomach wall (corpus)	1 95 ± 0 3	1.2	0 014	0 009
Skin	0 20 ± 0 03	0 12	0 054	0 042
Triceps muscle	0 20 ± 0 05	0 12	0 041	0 021

large real pores with average sizes varying from one tissue to another. If due entirely to pinocytosis one has to assume that there are different kinds of vesicles, some, P_{M}, transporting proteins with little size discrimination, others, P_{A}, causing marked sieving effects.

In a number of other tissues—again with continuous or fenestrated capillaries—the data cannot be explained by model 1. Here the turnover rate constant for albumin was higher than that for IgG. The ratio turnover rate of IgG/turnover rate of albumin was similar to that in the vascular beds described by model 1 or lower. So far model 2 A thus seems to describe the conditions in these tissues. But one has to consider also the extravascular space ratios. In all tissues in question the ratios were rather high. The question is then whether there is any indication for selective IgG accumulation in the tissues. A tentative answer to this question can be obtained by comparing the extravascular space ratios with known values for the lymph plasma ratios for the two proteins. In dog hearts the albumin C_{lymph}/C_{plasma} ratio is 0.74 and the corresponding value for globulin is 0.55 (see Courtice *et al.* 1974). The extravascular globulin/albumin space ratio expected if the tissue fluid had the same relative concentrations as the lymph then would be 0.55/0.74 0.74. The observed value for the extravascular IgG/albumin space ratio was 0.77. Thus if it is assumed that IgG concentrations are roughly proportional to total globulin concentrations, model 2 A seems to describe the situation in heart muscle. In the small intestine the extravascular space ratio expected from corresponding data in dogs is about 0.8 and in cats 0.9 (see Courtice *et al.* 1974). This is in reasonable agreement with the observed value, 0.93.

In the lung a recent study in sheep (Blake and Staub 1976) indicates that the extravascular space ratio should be 0.65. Taylor *et al.* (1967) obtained results in adult dogs indicating a value of 0.70 (in pups about 1.0). The value observed here, 0.94, thus is far above that expected in adult animals suggesting that in this tissue one has to consider also model 2 B.

Capillary permeability

Table I and II show more than 20-fold differences in the turnover rates of albumin and IgG between the different tissues. The results are in apparent agreement with the traditional concept that the protein permeability in fenestrated capillaries is higher than that in the

continuous capillaries. And heart muscle is an exception in having continuous capillar[illegible] a high protein permeability However if extraction values are compared this sugge[illegible] becomes highly questionable. Extraction of albumin, E_a, and IgG was defined as the turnover rate of the protein in question/regional plasma flow The outflux of album[illegible] through a capillary bed into the tissue depends on the permeability of the vessels t[illegible] albumin, P_a, and the surface of the capillaries, S, and the concentration of albumin i[illegible] plasma, C_{apl}.

$$J = P S C_{apl}$$

The amount of albumin entering the capillary bed with the blood depends on the pl[illegible] flow Q and the plasma concentration of albumin, C_{apl}

$$\text{Thus } E_a = \frac{P \ S \ C_{apl}}{Q \ C_{apl}} = \frac{P \ S}{Q}$$

The conditions for IgG are analogous. The extraction values, Table V in a number of th[illegible] were quite similar irrespective of differences in capillary ultrastructure, Table III Th[illegible] the plasma flow is related to the number of perfused capillaries and thereby to the capi[illegible] surface—which is the case if the average blood flow in the perfused capillaries is abou[illegible] same in the diffent tissues—it turns out that the protein permeability of the capillary wa[illegible] most tissues with continuous or fenestrated capillaries may be rather similar The bra[illegible] an obvious exception which will be discussed later The small intestine may also be an ex[illegible] tion having exctraction values 3–5 times those in most other tissues. And the lung is another exception In rabbits the plasma flow through the lung is about 60 ml/min/g (V[illegible] mers, Bartlett and Johnson 1976) With a turnover rate of 0.58 / l/min/g for albumin extraction value is 0 001 % which is less than 1/10 of that in most tissues where the bl[illegible] flow is adjusted to the needs of the tissue.

Approximative values for capillary surface have been reported for some tissues. In rab[illegible] capillary surface in heart and vasodilated skeletal muscle (Spector 1956) are 800 and 75 c[illegible] respectively In kidney cortex and medulla in dogs capillary surface areas are 350 c[illegible] and 780 cm /g respectively (Kügelgren and Braunger 1962) If these values are used values for albumin permeability in heart muscle, skeletal muscle, kidney cortex and med[illegible] are 160 70 34 and 26 µl/min/m capillary surface respectively For IgG the permeab[illegible] values are 55 33 33 and 16 µl/min/m respectively Thus the difference in turnover [illegible] between heart and skeletal muscle is easily accounted for by the difference in capillary [illegible] face. And there is no evidence in these data indicating that fenestrated capillaries in gen[illegible] have a markedly higher protein permeability than the nonfenestrated. Hence there is [illegible] indication for the presence of more large pores in fenestrated capillaries than in continu[illegible] capillaries.

As mentioned in the introduction no large real pores have ever been observed in c[illegible] tinuous capillaries and their presence in fenestrated capillaries is uncertain In fact, i[illegible] only in the small intestine (Simionescu, Simionescu and Palade 1972) that there is stro[illegible] evidence for passage of macromolecules through the fenestre. Pinocytotic vesicles on [illegible] other hand are present both in continuous and fenestrated capillaries and morphologi[illegible]

vidence indicates that in both kinds of capillaries they are involved in the transport of macro-molecules. The capillaries of the central nervous system represent an interesting exception pinocytosis being scarce and not involved in transport across the endothelial cells. And there the capillary permeability to protein is practically zero. One has to consider the possibility then that pinocytosis may account for most of the protein permeability in most fenestrated as well as in most continuous capillaries.

Studies with low molecular weight substances indicate that in skeletal muscle the functional small pores have permeability characteristics similar to those of water-filled pores with diameter of 7–8 nm (see Michel 1972). It seems likely then that in all model 1 tissues there is P_H and P pinocytosis and/or filtration through large pores resulting in an outflow of modified plasma and 7–8 nm pores (or equivalent slits) permitting ultrafiltration and diffusion of the low molecular weight substances.

In model 2 tissues albumin—and possibly also IgG—transport through the capillary wall was bidirectional. For two of these tissues, heart muscle and lung, studies with low molecular weight substances have indicated that the small pores have diameters of about 9 nm (see Michel 1972). Such pores would have a certain permeability to albumin (molecular (Stokes-Einstein) diameter 7 nm) but not to IgG (molecular diameter 11 nm). It is possible then that the main difference between model 1 and 2 tissues is that in the latter tissues there is appreciable diffusion of albumin through the small pore system. But diffusion through large pores also may contribute to model 2 behaviour.

Protein dynamics in tissue fluids

As discussed for model 2 B IgG may be more hindered than the albumin on its way to the lymph vessels. The restriction may be located in the tissue or in the walls of the lymph capillaries. In vitro experiments have indicated that the presence in connective tissue of glucosaminoglucanes might affect the plasma proteins passing through the tissue. Two types of interference have been discussed (Laurent 1970): one is that the tissue fluid has to pass through the gel which may cause filtration effects, the large molecules tending to move at a reduced rate through the gel, another possible interference is that the glucosaminoglucanes are present in discrete compartments with the tissue fluid passing in between. Such an arrangement might lead to a gel chromatography effect, the larger molecules passing at higher rates through the tissue than the smaller molecules. In the present experiments IgG had the same or a lower turnover rate constant than the albumin in all tissues permitting comparisons. In the tissues with the same turnover rate constants for the two proteins there was thus no observable interference of the glucosaminoglucanes with the protein dynamics. Once outside the capillaries the proteins appeared to pass through the tissue essentially with a bulk flow. Of course this might be a net result—slight gel chromatography effects cancelling weak filter effects and effects due to differences in diffusion coefficients.

In the lung with a lower turnover rate constant for IgG than for albumin and a space ratio markedly higher than expected from published lymph data, a combination of model 2 A and B may explain the results. It is not clear if filter effects in the tissue or in the walls of the lymph capillaries or adsorption of IgG to some tissue components was the mechanism causing the large IgG space.

continuous capillaries. And heart muscle is an exception in having continuous capillaries with a high protein permeability However if extraction values are compared this suggestion becomes highly questionable. Extraction of albumin, E_a and IgG was defined as the ratio turnover rate of the protein in question/regional plasma flow The outflux of albumin, J, through a capillary bed into the tissue depends on the permeability of the vessels to albumin, P_a, and the surface of the capillaries, S, and the concentration of albumin in plasma, C_{apl}.

$$J = P\ S\ C_{apl}$$

The amount of albumin entering the capillary bed with the blood depends on the plasma flow Q and the plasma concentration of albumin, C_{apl}.

$$\text{Thus } E_a = \frac{P\ S\ C_{apl}}{Q\ C_{apl}} = \frac{P\ S}{Q}$$

The conditions for IgG are analogous. The extraction values, Table V in a number of tissues were quite similar irrespective of differences in capillary ultrastructure, Table III. Thus if the plasma flow is related to the number of perfused capillaries and thereby to the capillary surface—which is the case if the average blood flow in the perfused capillaries is about the same in the diffent tissues—it turns out that the protein permeability of the capillary wall in most tissues with continuous or fenestrated capillaries may be rather similar The brain is an obvious exception which will be discussed later The small intestine may also be an exception having exctraction values 3–5 times those in most other tissues. And the lung is another exception. In rabbits the plasma flow through the lung is about 60 ml/min/g (Wimmers, Bartlett and Johnson 1976) With a turnover rate of 0.58 μl/min/g for albumin the extraction value is 0 001 % which is less than 1/10 of that in most tissues where the blood flow is adjusted to the needs of the tissue.

Approximative values for capillary surface have been reported for some tissues. In rabbits capillary surface in heart and vasodilated skeletal muscle (Spector 1956) are 800 and 75 cm²/g respectively In kidney cortex and medulla in dogs capillary surface areas are 350 cm²/g and 780 cm /g respectively (Kügelgren and Braunger 1962) If these values are used the values for albumin permeability in heart muscle, skeletal muscle, kidney cortex and medulla are 160 70, 34 and 26 μl/min/m capillary surface, respectively For IgG the permeability values are 55 33 33 and 16 μl/min/m respectively Thus the difference in turnover rate between heart and skeletal muscle is easily accounted for by the difference in capillary surface. And there is no evidence in these data indicating that fenestrated capillaries in general have a markedly higher protein permeability than the nonfenestrated Hence there is no indication for the presence of more large pores in fenestrated capillaries than in continuous capillaries.

As mentioned in the introduction no large real pores have ever been observed in continuous capillaries and their presence in fenestrated capillaries is uncertain In fact, it is only in the small intestine (Simionescu, Simionescu and Palade 1972) that there is strong evidence for passage of macromolecules through the fenestre. Pinocytotic vesicles on the other hand are present both in continuous and fenestrated capillaries and morphologic

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Adsorption of IgG

Anatomical studies have shown that in the liver spleen (see Bennet *et al* 1959), adrenals (Mori and Onoé 1967) and bone marrow (Majno 1966) there are gap capillaries. In the expts. reported here the movement of albumin and IgG out of the blood vessels was so rapid that a turnover rate could not be calculated for any of these tissues. In the liver spleen and adrenals but not in the bone marrow the IgG space was larger than the albumin space at all times. This was a very unexpected result—first observed in preliminary experiments with human IgG. It seemed possible that heterologous or denaturated IgG might be accumulated in the reticuloendothelial cells of the three tissues and for this reason screened rabbit IgG was used. However even then there was accumulation of IgG Evidence for transient binding of homologous IgG to macrophages obtained from the lung was reported by Phillips-Quagliata *et al* (1971) The present results suggest that there may be a similar phenomenon in the liver spleen and adrenals.

The drainage of tissue fluid

Model 1 as well as 2 A and B assume that there is protein drainage from the tissues by lymph flow There are two tissues where this does not apply the choroid plexus and the kidney medulla. In these tissues there are no lymph vessels. The routes by which the extravascular proteins leave the choroid plexus are not completely known but drainage into the ventricular fluid is one obvious mechanism that has been considered (Brightman, Reese and Feder 1970). The following calculation suggests that such drainage is the main mechanism. In rabbits the rate of formation of the cerebrospinal fluid is about 10 µl/min (Welch 1963 Bradbury and Davson 1964) The weight of the choroid plexus in the expts. reported here was about 15 mg. The turnover rate of the albumin corresponded to about 0.9 µl plasma equivalent fluid per min per gram tissue or 0 014 µl per min for the whole plexus. If all the albumin were drained into the cerebrospinal fluid the albumin concentration of this fluid would be about 0 14% of that in the plasma. This figure is even less than the mean figure that was reported by Amtorp and Sorensen (1974) In their study the albumin concentration in the plasma was about 40 g/l and that in the aqueous humor about 0.12 g/l The concentration of albumin in the cerebrospinal fluid thus was about 0 3% of that in the plasma.

In the kidney lymph vessels are present in the cortex but not in the medulla (Kriz and Dieterich 1970) It is paradoxical that the tissue in table 1 having the fastest turnover rate of albumin lacks lymph vessels. At present nothing seems to be known about the drainage of tissue fluid from the medulla. One can only speculate that there may be a sluggish flow of tissue fluid into the lymph vessels of the cortex.

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Influence of Age on Sensitivity and Effector Mechanisms of the Carotid Baroreflex

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Abstract

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In 30 healthy subjects aged 20–48 years the haemodynamic response to carotid sinus stimulation (neck suction 40 mmHg) was studied. Heart rate, arterial pressure and cardiac output (dye dilution technique) were measured. In order to evaluate the effect of ge on carotid sinus function the material was subdivided into two arbitrary subgroups, aged up to 30 years (= 15) respectively 30 years and bove (= 15). Carotid sinus stimulation induced significantly greater reduction in mean arterial pressure in the younger group compared to the older group. The heart rate reduction as, on the average, slightly greater in the younger group though the difference was not significant. In both groups significant decrease in cardiac output contributed to the demonstrated reduction in mean arterial pressure. As the decrease in cardiac output was, on the average, slightly smaller in the younger group, the results indicate that the greater blood pressure response in the younger group was due to greater reduction in peripheral vascular resistance. This is further supported by the finding of significant correlation between changes in total peripheral vascular resistance, elicited by carotid sinus stimulation and age.

Key words: Carotid sinus, pressor receptors, age factors, cardiac output, blood pressure, heart rate, vascular resistance

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An inverse relation between age and baroreceptor function has been demonstrated in four recent articles (Bristow and Honour 1969 Eckberg, Drabinsky and Braunwald 1971 Gribbin *et al.* 1971 and Duke *et al.* 1976). I these studies the baroreflex was tested by the Valsalv maneuver and/or pharmacologically (angiotensin or phenylephrine) induced blood pressure increase Quantitatively the baroreflex has usually been evaluated from the decrease in heart rate elicited by the induced increase in blood pressure as suggested by Smyth, Sleight and Pickering (1969). However the primary function of the baroreceptors are to regulate blood pressure. An unimpaired blood pressure regulating capacity of the carotid sinus can be found during conditions when the effect on heart ate is virtually abolished (Bevegård *et al.* 1977 b) With this background w considered it of interest to study the baroreceptor effect on heart rate and blood pressure separately to further evaluate the effect of age on baroreceptor function.

TABLE I Dimensional data. Mean values ± S.E. I age < 30 years, n = 15 II age > 30 years, n 15 BSA Body surface area from nomogram of Dubois and Dubois (1916).

	Age (years)	Weight (kg)	Height (m)	BSA (m^2)
I	24.8 ± 0 81	75 7 ± 2.20	1 79 ± 0.02	1 95 ± 0 04
II	36.9 ± 1 71	78 0 ± 2.94	1 83 ± 0.01	2.00 ± 0 04
Diff	+ 12.1	+ 2.3	+ 0 04	+ 0 05
Sign of diff	p < 0.001	n s.	n.s.	n s.

There is disagreement in literature about the extent to which a decrease in cardiac outpt and/or a reduction in total peripheral vascular resistance contributes to the blood pressu decrease during baroreceptor stimulation (for ref see Kirscheim 1975). This has also bee discussed in some detail in an earlier article (Bevegård, Castenfors and Lindblad 1977 c Therefore we have also tried to elucidate whether age is a factor influencing the relativ importance of the two effector mechanisms of the carotid sinus, contributing to the dis crepancy stated above Such data might be of interest since most data in normals refer t young volunteers, whereas data on patients, for example with hypertension or angina pector usually refer to distinctly older subjects

Material

Data from 30 healthy male volu teers (blood donors) are presented 20 of the subjects participated i other studies (Bevegård *et al* 1977 c, d). The material was arbitrarily divided accordi g to age in 2 equa sized groups Dimensional data for the two groups are given T ble I

Methods

Carotid sinus st mulation

The carotid arterial stretch receptors were stimulated by pplying subatmospheric pressure (−40 mmHg rou d the neck Details of the stimulation equipment and methods are presented elsewhere (Bevegård *et al* 1977 b,).

H mod ynamic measurement

Details consideri g catheterization procedure, measurements f mean rterial blood pressure (MAP) heart rate (HR) and cardiac output ($\dot{Q}$) re given elsewere (Bevegård *et al* 1977 a) To minimize difference due to dimensional factors ca diac output, strok volume and total peripheral vascula resistance (MAP divided by $\dot{Q}$) are corrected for body surface rea (BSA) and presented as cardiac index (CI), strok inde (SVI) and vascula resistance index (TPI) respect vely

Statistical methods

Details are given elsewhere (Bevegård *et al.* 1977).

General xperimental design

Heart rate, blood pressure and cardiac ndex were compared duri g resti g cond tion nd during neck suction The dye injection for determination of cardiac output was made ppro imately mm after the beginning of neck suction. The heart rat presented represents an erage d ring the indicator curv T obta n comparable nd complet data in all s bjects only blood pressures immediately prior to cardiac output determination have been used for calculation.

TABLE II. Some haemodynamic data during control condition. Mean values ± S.E. I, age 30 years, n = 15. II, age > 30 years, n = 15.

	HR	MAP	CI	SVI	TPI
I	69.3 (±2.24)	95.7 (±2.06)	3.5 (±0.20)	50 (±2.6)	7.8 (±0.62)
II	60.7 (±1.93)	95.3 (±1.79)	3.3 (±0.19)	57 (±2.40)	7.3 (±0.39)
Difference	8.6	−0.4	0	+5	−0.5
Sign. of diff.	p 0.01	n.s.			n.s.

Results

Hemodynamic variables during control condition

During control condition (Table II) the younger (I) and the older group (II) showed essentially the same values considering mean arterial pressure, cardiac index, stroke index and total peripheral vascular resistance index whereas the heart rate was slightly higher in the younger group.

Hemodynamic response to stimulation of the carotid sinus

In both groups (Table III) neck suction induced a significant decrease in heart rate, mean arterial pressure and cardiac index, but only in the older group (II) did the reduction in stroke index reach significant level. Total peripheral vascular resistance index decreased significantly in the younger group (I) whereas on the average a minor but not significant increase was found in the older group (II). On comparison of the two groups (Table III) the reduction in mean arterial pressure was significantly greater in the younger group (I), compared to the older group (II). The difference in reaction regarding total peripheral vascular resistance index between the two groups is significant. On the average the heart rate decrease was slightly greater in the younger group whereas the decrease in cardiac index and stroke index were slightly smaller in the younger group though these differences did not reach significant levels.

Changes in total peripheral vascular resistance index in relation to age

On correlating the change in total peripheral vascular resistance index to the age of the subjects (Fig. 1) a significant correlation (r = 0.61, p 0.001) is found.

TABLE III. Effect of baroreceptor stimulation on heart rate (HR), mean arterial pressure (MAP), cardiac index (CI), stroke index (SVI) and vascular resistance index (TPI). Mean values ± S.E. I, age 30 years, 15. II, age 30 years, n = 15.

Variable	I		II		I–II
	Change during stimulation	Sign. of difference	Change during stimulation	Sign. of difference	Sign. of difference
Δ HR	−3.3 (±0.73)	p<0.001	−2.1 (±0.56)	p<0.01	s.
Δ MAP	11.5 (±1.45)	p 0.001	−6.9 (±1.55)	p<0.001	p<0.05
Δ CI	0.3 (±0.09)	p 0.05	0.4 (±0.07)	p 0.001	n.s.
Δ SVI	1 (±1.07)		−4 (±1.02)	p<0.01	
Δ TPI	0.4 (±0.18)	p<0.05	+0.2 (±0.14)		p<0.01

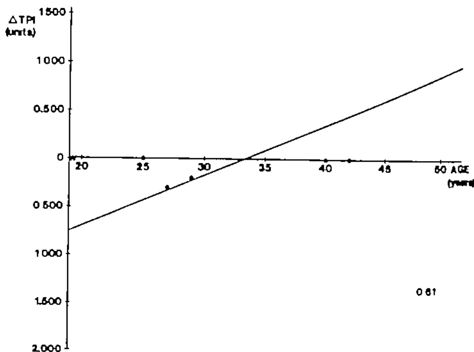


Fig. 1 Correlation between age and change in TPI induced by stimulation of the carotid sinus. TPI = peripheral vascular resistance per square metre body surface area.

Discussion

The function of the arterial baroreceptors has mainly been evaluated by four different methods in man 1) studying the response to a Valsalva maneuver 2) studying the cardiac slowing in response to a pharmacologically induced pressure increase, 3) neck suction and 4) electrical stimulation of the carotid sinus. The latter method has, however been used only in elderly patients (Carlsten *et al* 1958 Tuckman *et al* 1966, Epstein *et al* 1969 Farrehi 1972) and thus no correlation with age has been possible.

Studies using the Valsalva maneuver technique (Appenzeller and Descarris 1964 Wei *et al* 1970 Gross 1976) suggest that baroreceptor function decreases with age. However as emphasized by Duke *et al* 1976 "the Valsalva maneuver test as a measure of intact baroreceptor mechanisms must be interpreted with caution" as it requires the active participation and co-operation of the subject The Valsalva maneuver can be suspected to be a rather complex stimulus affecting intrathoracic receptors as well as the arterial stretch receptors in the carotid sinus.

Testing the arterial baroreceptors by pharmacologically induced blood pressure increase has been used repeatedly as a quantitative measure to show that baroreceptor function is altered in various situations in man. The method has been evaluated in detail by Smyth *et al* (1969) and Bristow *et al* (1969) With this stimulus a negative relation between age and baroreceptor function has been demonstrated by Bristow *et al* (1969) Eckberg *et al* (1971) and Duke *et al* (1976) Using pharmacologically induced changes in blood pressure Korner *et al* (1974) demonstrated a diminished baroreceptor heart rate response in older subjects using similar arbitrary subgroups as in this study However as emphasized by Duke

et al. (1976) the pressor test describes baroreceptor function only in terms of cardiac slowing and tells very little about the mechanisms involved. Using this stimulus it is not possible to separate the baroreceptor effect on heart rate and blood pressure.

Neck suction is a stimulus primarily affecting only the carotid sinus, but as emphasized by for example Burton (1965) it is an unphysiological approach as the error signal—an increased blood pressure—is reaching the brain from only one receptor area. Neck suction has been used by several authors (for ref. see Bevegård *et al.* 1977 b) to quantitatively study the carotid buffer reflex. Using this stimulation method, Wagner, Wackerbauer and Hilger (1968) in 26 normal subjects, covering the rather narrow range 23–36 years found no influence of age on the heart rate response of the carotid sinus. The present data demonstrate a greater blood pressure regulating capacity in the younger subjects compared to the older subjects. Considering mean values a similar tendency is found regarding heart rate though the difference did not reach significant level. In this material a correlation, though weak, ($r = 0.4$, $p < 0.05$) is found between changes in mean arterial blood pressure and heart rate induced by neck suction. *The present findings thus are in agreement with results earlier* demonstrated using the pressor test.

On dividing the material into arbitrary subgroups according to chronological age, as a substitute for biological age, the individual responses demonstrate a considerable scatter within the two groups. Age is only one of the factors known to influence baroreceptor function. With a similar technique as in this study Stegemann, Busert and Brock (1974) have demonstrated a reduced baroreceptor function in physically well trained subjects. A dependence of intra-thoracic blood volume on the carotid sinus reflex has also been demonstrated (Bevegård *et al.* 1977 b).

Findings in the literature disagree about the relative importance of the two effector mechanisms of the arterial baroreceptors, changes in cardiac output and vascular resistance.

As summarized in an earlier article (Bevegård *et al.* 1977 c) this lack of agreement applies also to studies in man. The findings in this study of a significantly greater reduction in mean arterial pressure in the younger group (I) in spite of an, on the mean, slightly smaller reduction in cardiac index compared to the older group (II), indicate that the reduction in vascular resistance index is greater in the younger group. An age dependent influence on the effect of the resistance vessels exerted by the carotid sinus can also be supported by the finding of a significant correlation between age and the changes in total peripheral vascular resistance index induced by baroreceptor stimulation (Fig. 1).

The effect of age may contribute to the disagreement about the relative importance of the two effector mechanisms stated above. As emphasized by Aars (1975) independent reductions in baroreflex sensitivity with age and hypertension should be taken into acount when planning clinical investigations.

Degenerative structural changes with ageing and "stiffening" of the arterial wall have been well documented (Blumenthal, Lansing and Grey 1950, Roach and Burton 1959, Learoyd and Taylor 1966 and O'Rourke *et al.* 1968).

An increased resistance to deformation could result in less deformation of the arterial wall at the receptor site and hence less baroreceptor stimulus for a given pressure decrease outside the arterial wall. It also seems possible that loss of elasticity in the arterioles results

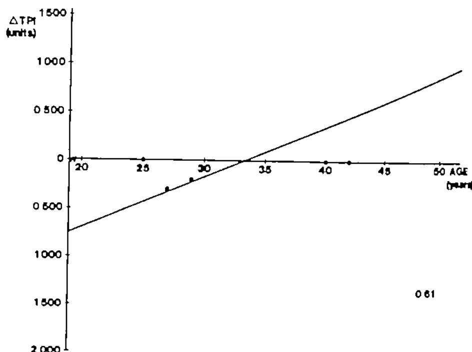


Fig 1 Correlation between age and change in TPI induced by stimulation of the carotid sinus. TPI total peripheral vascular resistance per square metre body surface area.

Discussion

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in a lesser change in vascular tone in response to the same change in neurogenic constric tone. Also an influence of age on the central nervous control of the baroreceptor sys and/or peripheral nervous system might contribute to the findings.

In conclusion the results indicate that the carotid sinus induced hemodynamic changes influenced by the age of the subject. This seems to be true considering both the magnit of blood pressure response as well as considering the relative importance of the two effec mechanisms—reductions in cardiac output and total peripheral vascular resistance.

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These findings motivated the present study to evaluate the relative importance of the two blood pressure reducing mechanisms on stimulation of the carotid sinus—reduction in cardiac output and reduction in total peripheral vascular resistance—under conditions with normal and reduced central blood volume.

Material and methods

Subjects. 10 healthy male volunteers (blood donors) participated. Mean values ± S.D. were for age 29.6 ± 3.9 years, for height 1.82 ± 0.06 m and for weight 80.4 ± 9.3 kg. A separate study of the heart rate response was made in six healthy subjects, mean values ± S.D. were for age 30.0 years ± 6.5 years, for height 1.83 ± 0.04 m and for body weight 72.2 ± 6.9 kg.

Carotid sinus stimulation. The carotid arterial stretch receptors were stimulated by varying the pressure in a plexiglass box enclosing the neck air-tightly. The pressure was lowered rapidly from atmospheric to −40 mmHg and kept constant during the stimulation period. Details of the stimulation equipment are presented elsewhere (Bevegård *et al.* 1977 b).

Lower body negative pressure. The lower body (below the iliac crest) was enclosed in an air-tight box. With the aid of a high capacity electric pump, the pressure in the box could be lowered rapidly to 40 mmHg below ambient and kept constant during the stimulation period. This procedure will be referred to as lower body negative pressure (LBNP −40). For technical details see Bevegård, Castenfors and Lindblad (1977).

Catheterization procedures and methods. Details considering statistical methods, catheterization procedures, measurements of mean arterial pressure (MAP), central venous pressure (CVP), heart rate (HR), cardiac output ($\dot{Q}$) and forearm blood flow (FBF) are given elsewhere (Bevegård *et al.* 1977). Total peripheral vascular resistance (TPVR) and regional vascular resistance (RVR) are calculated as MAP divided by $\dot{Q}$ respectively forearm blood flow (FBF) and expressed in arbitrary units.

General experimental design. The subjects participated in an initial twelve-minute experimental run focusing on the dynamic features of the carotid baroreflex during gradually increasing levels of LBNP. These data are presented in a separate article (Bevegård *et al.* 1977 b). After re-gaining stable conditions, estimated from heart rate and blood pressure, the experimental scheme summarized in Fig. 1 was commenced. Cardiac output ($\dot{Q}$) and forearm blood flow (FBF) determinations were made as indicated by the heavy lines (Fig. 1). The heart rate (HR) presented under Results represents the mean value for the period when cardiac output was determined. Intravascular pressures were measured in the intervals between $\dot{Q}$ determinations. The values presented are an average of measurements immediately before and after the determination of $\dot{Q}$.

In two subjects $\dot{Q}$ was determined not only before and during but also after carotid sinus stimulation during continuous LBNP. In these two subjects the LBNP period was extended approximately 5 min.

In 6 subjects a separate study was made of the heart rate response to the same baroreceptor stimulus under control conditions, LBNP −40 and 60° head up tilt. T illustrate the time sequence of the heart rate response the heart rate was averaged in fifteen second periods.

Results

Circulatory effects of LBNP −40. Table I gives the values collected at $\dot{Q}$ and $\dot{Q}$ according to Fig. 1. Compared with control conditions, LBNP −40 induced a significant decrease in central venous pressure, whereas mean arterial pressure was essentially unchanged. Heart rate increased 5.2 beats min which was not enough to compensate for a decrease of stroke volume by 26 ml, thus $\dot{Q}$ on the average decreased 1.3 l min^{-1}. Total peripheral vascular resistance and regional vascular resistance both increased significantly.

Hemodynamic response to stimulation of the carotid sinus

Control conditions (Table II A presents the differences between the values collected at $\dot{Q}_1$ and $\dot{Q}_2$ according to Fig. 1). Neck suction induced a significant reduction in mean arterial

Acta physiol. scand. 1977 101 50-57

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Effect of Carotid Sinus Stimulation on Cardiac Output and Peripheral Vascular Resistance during Changes in Blood Volume Distribution in Man

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Abstract

BEVEGÅRD S., J CASTENFORS and L E. LINDBLAD *Effect of carotid sinus stimulation o cardiac output and peripheral vascular resistance during changes in blood volume distrib tion in man* Acta physiol scand. 1977 *101* 50-57

In ten healthy subjects (mean age 29 6 years) the hemodynamic response to carotid sinus stimulation (ne suction −40 mmHg) was studied under control conditions and during peripheral pooling of blood (low body negative pressure) Heart rate, arterial and central venous pressure, card ac output and forearm bl flow were measured The time sequence of the heart rate response was studied separately in six healt subjects. During control conditions, carotid sinus stimulation oduced a significant decrease in arter pressure and heart rate. The blood pressure decrease mainly reflected reduction in cardiac output, to peripheral vascular resistance being essentially nchanged. However in the skeletal muscle, represen by a forearm segment vascular resistance decreased significantly During lower body negative press (LBNP) the same stim lation of the carotid sinus induced a significantly greater fall in mean arterial pre sure even though the reduction in cardiac output was slightly smaller on the average than n the contr cond tion. *The heart rat increased probably secondary to time dependent increase in heart rate dict* by the conti uous LBNP stimulus. Total peripheral vascular resistance decreased significantly durin LBNP the reaction likewise differing significantly from that n the control condition. Thus the augmen blood pressure response was due t a more pronounced vasodilatation when the carotid sinus as stir ulated during lower body negative pressure. The results indicate that the hemodynamic changes dicta by carotid sin stimulatio are modified by cha ges the distribution of blood vol me and in the ton of resistance vessels.

K y words. Carotid sinus, blood volume, central venous pressure, pressor receptors, cardiac output, bloo pressure, vascular resist nce

Previous *studies (Bevegård et al* 1977 b) have *demonstrated that the effect of a standardize* baroreceptor stimulus (sinusoidal changes in the pressure around the neck) on heart rat and blood pressure is modified by changes in blood volume distribution, possibly via stim ulation of intrathoracic low pressure receptors. Thus, during lower body negative pressur (−40 mmHg) the blood pressure regulating capacity of the carotid sinus is augmented while the heart rate response *is distorted and difficult to interpret.*

ble II. Hemodynamic response to baroreceptor stimulation under control conditions (A) and lower body negative pressure (LBNP) (B) (=10). Mean values ± S.E.

	A		B		A–B
ean arterial pressure (mmHg)	−8.5 ±1.7	p 0.01	−13.3 ±1.2	p<0.001	p<0.01
rdiac output (l·min⁻¹)	−0.7 ±0.2	p 0.01	−0.4 ±0.1	p<0.05	s.
eart rate (beats min⁻¹)	3.0 ±1.0	p 0.05	+5.2 ±0.8	p<0.001	p<0.001
troke volume (ml)	−8.1 ±2.4	p<0.05	11.7 ±1.9	p<0.001	s.
otal peripheral vascular resistance (mmu)	+0.1 ±0.4	n.s.	1.2 ±0.4	p<0.05	p<0.01
orearm blood flow (ml·min⁻¹ 100 ml⁻¹)	+0.2 ±0.2	s.	0.0 ±0.3	s.	
tegonal vascular resistance (mmu)	7.1 ±2.5	p<0.05	−7.9 ±2.6	p 0.05	s.

n cardiac output during LBNP compared with control conditions, −0.4 l min⁻¹ and −0.7 l min⁻¹ respectively the difference not being significant. In two cases, as described, the LBNP period was extended so that cardiac output could also be determined after the end of neck suction. In both subjects cardiac output increased after the end of neck suction, in spite of persistent LBNP stimulation (Fig. 2).

The heart rate response also differed significantly between the two conditions, changing from a decrease in the control period to an increase during LBNP. The time sequence of the heart rate response during LBNP (Fig. 3) suggests that this is due to a gradual time-dependent increase in heart rate, interrupted by a transient decrease in heart rate shortly after the application of neck suction.

Time sequence of heart rate response to neck suction under control conditions, LBNP 40 and head-up tilt (60°) (Fig. 4)

Under control conditions there was an immediate significant decrease in heart rate which sustained during the stimulation period. During LBNP and head-up tilt 60° there was a less marked and also a less well sustained decrease in heart rate, especially in the tilt condition.

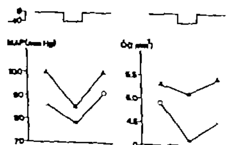


Fig. 2. Mean arterial pressure (MAP) and cardiac output (Q) in two subjects before, during and after neck suction and simultaneous lower body negative pressure (LBNP).

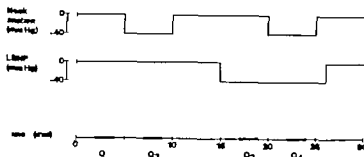


Fig. 1 General experimental design. Heavy lines indicate the time when cardiac output and forearm blood flow was measured. (LBNP = lower body negative pressure)

pressure as well as in heart rate cardiac output and stroke volume. Total peripheral vascular resistance was essentially unchanged, whereas regional vascular resistance in the forearm decreased significantly

LBNP −40 (Table II B presents the differences between values collected at Q̇ and Q̇ according to Fig. 1) Also during LBNP neck suction induced a significant decrease in mean arterial pressure, cardiac output and stroke volume, whereas heart rate increased significantly Total peripheral vascular resistance and regional vascular resistance both decreased significantly

Hemodynamic response of neck suction during LBNP compared to control conditions (Table II A–B)

Compared with control conditions, neck suction elicited a significantly greater reduction in mean arterial pressure during LBNP The significant decrease in total peripheral vascular resistance during LBNP differed significantly from the reaction found during control conditions, whereas regional vascular resistance in the forearm showed essentially the same significant decrease under both conditions. On the average there was a less marked decrease

TABLE I Effect of lower body negative pressure −40 mmHg (LBNP −40) on some hemodynamic variables (n = 10). Mean values ± S.E.

	Control	*LBNP −40*	*Diff.*	*Sign. of diff*
Mean arterial pressure (mmHg)	95.5 ±1.8	96.8 ±1 6	+1.3 ±0.9	n.s.
Central venous pressure (mmHg)	7.9 ±0.6	3 4 ±0.6	−4 4 ±0.5	$p < 0.001$
Cardiac output (l min^{-1})	6.6 ±0 4	5 4 ±0.3	−1.3 ±0.2	$p < 0.001$
Heart rat (beats min^{-1})	64 0 ±2.7	69.2 ±3.3	+5.2 ±1.9	$p < 0.05$
Stroke volume (ml)	106 ±4	79 ±3	−[illegible]6 ±3	$p < 0.001$
Total peripheral vascular resistance (units)	14.7 ±0.7	18.3 ±0.8	+3.6 ±0.4	$p < 0.001$
F rearm blood flow (ml min^{-1} 100 ml^{-1})	2.9 ±0.4	1.9 ±0.2	−1.0 ±0.[illegible]	$p < 0.01$
Regional vascular resistance (units)	36.8 ±3.5	54 7 ±4.9	+17.9 ±2.2	$p < 0.001$

TABLE II. Hemodynamic responses to baroreceptor stimulation under control conditions (A) and lower body negative pressure (LBNP) (B) (n = 10). Mean values ± S.E.

	A		B		A–B
Mean arterial pressure (mmHg)	−8.5 ±1.7	$p<0.01$	−13.3 ±1.2	p 0.001	p 0.01
Cardiac output (l min⁻¹)	−0.7 ±0.2	$p<0.01$	−0.4 ±0.1	$p<0.05$	s.
Heart rate (beats min⁻¹)	−3.0 ±1.0	$p<0.05$	+5.2 ±0.8	p 0.001	p 0.001
Stroke volume (ml)	−8.1 ±2.4	$p<0.05$	−11.7 ±1.9	p 0.001	s.
Total peripheral vascular resistance (units)	+0.1 ±0.4	s.	−1.2 ±0.4	p 0.05	p 0.01
Forearm blood flow (ml min⁻¹ 100 ml⁻¹)	0.2 ±0.2	s.	0.0 ±0.3	s.	s.
Regional vascular resistance (units)	7.1 ±2.5	p 0.05	−7.9 ±2.6	$p<0.05$	n.s.

in cardiac output during LBNP compared with control conditions, −0.4 l min⁻¹ and −0.7 l min⁻¹ respectively the difference not being significant. In two cases, as described, the LBNP period was extended so that cardiac output could also be determined after the end of neck suction. In both subjects cardiac output increased after the end of neck suction, in spite of persistent LBNP stimulation (Fig. 2).

The heart rate response also differed significantly between the two conditions, changing from a decrease in the control period to an increase during LBNP. The time sequence of the heart rate response during LBNP (Fig. 3) suggests that this is due to a gradual time-dependent increase in heart rate, interrupted by a transient decrease in heart rate shortly after the application of neck suction.

Time sequence of heart rate response to neck suction under control conditions, LBNP 40 and head-up tilt (60°) (Fig. 4)

Under control conditions there was an immediate significant decrease in heart rate which sustained during the stimulation period. During LBNP and head-up tilt 60° there was a less marked and also less well sustained decrease in heart rate, especially in the tilt condition.

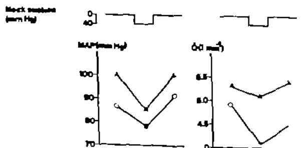


Fig. 2. Mean arterial pressure (MAP) and cardiac output (Q) in two subjects before, during and after neck suction and simultaneous lower body negative pressure (LBNP).

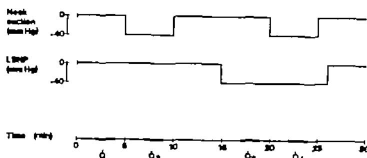


Fig. 1 General experimental design. Heavy lines indicate the time when cardiac output and forearm blood flow was measured. (LBNP = lower body negative pressure.)

pressure as well as in heart rate, cardiac output and stroke volume. Total peripheral vascular resistance was essentially unchanged whereas regional vascular resistance in the forearm decreased significantly

LBNP −40 (Table II B presents the differences between values collected at $\dot{Q}$ and $\dot{Q}$ according to Fig. 1) Also during LBNP neck suction induced a significant decrease in mean arterial pressure, cardiac output and stroke volume, whereas heart rate increased significantly Total peripheral vascular resistance and regional vascular resistance both decreased significantly

Hemodynamic response of neck suction during LBNP compared to control conditions (Table II A–B)

Compared with control conditions, neck suction elicited a significantly greater reduction in mean arterial pressure during LBNP The significant decrease in total peripheral vascular resistance during LBNP differed significantly from the reaction found during control conditions, whereas regional vascular resistance in the forearm showed essentially the same significant decrease under both conditions. On the average there was a less marked decrease

TABLE I Effect of lower body negative pressure −40 mmHg (LBNP −40) on some hemodynamic variables (=10) Mean values ± S.E.

	Control	LBNP −40	Diff	Sign. of diff.
Mean arterial pressure (mmHg)	95.5 ±1.8	96.8 ±1 6	+1.3 ±0.9	n.s.
Central venous pressure (mmHg)	7.9 ±0.6	3.4 ±0.6	−4 4 ±0.5	p < 0.001
Cardiac output (l min⁻¹)	6.6 ±0.4	5.4 ±0.3	−1.3 ±0.2	p 0 001
Heart rate (beats min⁻¹)	64 0 ±2.7	69 2 ±3.3	+5.2 ±1.9	p < 0 05
Stroke volume (ml)	106 ±4	79 ±3	−26 ±3	p < 0.001
Total peripheral vascular resistance (units)	14 7 ±0.7	18.3 ±0.8	+3.6 ±0.4	p < 0 001
Forearm blood flow (ml min⁻¹ 100 ml⁻¹)	2.9 ±0.4	1 9 ±0.2	−1.0 ±0 2	p < 0.01
Regional vascular resistance (units)	36.8 ±3.5	54 7 ±4 9	+17.9 ±2.2	p < 0.001

n cardiac output contributes to the blood pressure reduction on stimulation of the carotid sinus (for ref see Kirchheim 1976). This lack of agreement applies even to studies in man. Using electrical stimulation of the carotid sinus nerve, Carlsten *et al* (1958) reported that patients under general anaesthesia displayed bradycardia and a decreased pulse pressure, suggesting a reduction in cardiac output. In conscious subjects using a similar stimulation technique, moderate decreases in cardiac output were demonstrated by Tuckman *et al* (1966) in subjects with severe hypertension and by Epstein *et al.* (1969) and Farrehi (1972) in subjects with coronary artery disease. However their results are difficult to compare with findings in healthy subjects. In six healthy subjects Bevegård and Shepherd (1966) found that cardiac output fell 0.6 l min^{-1} when the pressure around the neck was reduced 40 mmHg. In twenty-three hypertensive subjects (mean age 43 years), Castenfors, Bevegård and Danielson (1976) reported that the same stimulus elicited a significant reduction in cardiac output (0.7 l min^{-1}).

On the other hand, Ernsting and Parry (1957), using the same stimulus in two subjects, found no significant difference in cardiac output. Recently Bjurstedt *et al* (1975) using a similar stimulus (subatmospheric pressure 40 mmHg in a plexiglass helmet enclosing the whole head) in eight healthy subjects reported a slight average increase (0.3 l min), though the change was not significant. The reduction in mean arterial pressure was due to significant decrease in total peripheral vascular resistance. This discrepancy may be due, at least in part, to the fact that their subjects were younger (mean age 24.8 years) than those in the present study A separate presentation of the influence of age on sensitivity and effector mechanisms of the carotid baroreflex in the extended age range 20–50 years reveals an age dependence of the relative importance of reductions in cardiac output and total peripheral vascular resistance respectively (Lindblad, 1977). A decrease in total peripheral vascular resistance is thus mainly found in younger subjects.

In a separate study (Bevegård *et al* 1977 b) using sinusoidally varying pressure around the neck to obtain standardized carotid sinus stimulus suitable for harmonic analysis, the results indicated an augmented blood pressure regulating capacity during LBNP The present study confirms this finding

The present data show the expected heart rate decrease during control conditions but an increase in heart rate during LBNP: this increase is probably at least partly related to the LBNP stimulus per se, since heart rate increased during the continuous LBNP stimulus (Fig. 3). When studying the heart rate response to neck suction during orthostatic stimulation, accomplished through head-up tilt or simulated through LBNP it is necessary to allow for a base line drift (Fig. 3 Fig. 4). Taking this drift into account, the baroreceptor stimulation seems to have induced a small and non-significant decrease in heart rate during LBNP as well as during tilt. This agrees with the findings during LBNP in the study mentioned earlier using sinusoidal variation of the pressure around the neck (Bevegård *et al* 1977 b). A decreased vagal tone during LBNP might be of importance for the reduced heart rate response (Eckberg, Fletcher and Braunwald 1972). The reduction in cardiac output was smaller during LBNP than during control conditions. To verify that the small reduction in cardiac output on neck suction during LBNP was due to neck suction and did not just represent time dependent gradual decrease during the continuous LBNP stimulus, cardiac output was

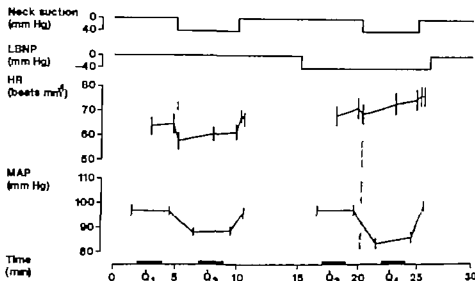


Fig. 3 Time sequence of the heart rate and blood pressure response to neck suction under control conditions and lower body negative pressure (LBNP). Mean values ± S.E. Sufficient data for this plot were available in only eight of the subjects.

Discussion

The hemodynamic effects of LBNP −40 have been discussed in a separate study (Bevegård *et al* 1977 a) where the results were similar to those found in the present material. As discussed in the earlier report, it is probable that reflexes originating in the low pressure area at least contribute to the demonstrated vasoconstriction, resulting in the essentially unchanged mean arterial pressure in spite of the demonstrated reduction in cardiac output.

The reflexly induced decrease in heart rate and reduction in blood pressure during carotid stimulation by neck suction are well known (Ernsting and Parry 1957 Bevegård and Shepherd 1966, Thron *et al* 1967 Wagner Wacherbauer and Hilger 1968, Beiser *et al* 1969 Stegmann, Busert and Brock 1974 Bjurstedt, Rosenhamer and Tydén 1975 Abboud *et al* 1975).

In this material baroreceptor stimulation during control conditions resulted in a significant reduction in cardiac output by 0 7 l min and essentially unchanged total peripheral vascular resistance. Findings in the literature disagree about the extent to which a decrease

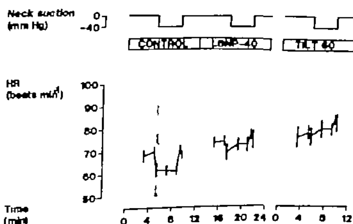


Fig. 4 Time sequence of heart rate response to neck suction during control conditions, lower body negative pressure −40 mmHg (LBNP −40) and head-up tilt (n = 6) Mean values ± S.E.

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determined in two cases before, during and directly after neck suction (Fig. 2). In these t subjects, no indication of a gradual time-dependent drop in cardiac output was found duri the period studied

The finding that LBNP stimulation augmented the blood pressure reduction during ne suction in spite of a smaller reduction in cardiac output indicates that the decrease in periphe vascular resistance is greater under this condition This is supported by the finding that t reduction in total peripheral vascular resistance during neck suction was significant o during LBNP

In the skeletal muscle (represented by a forearm segment) the finding of an unchang forearm blood flow during neck suction in spite of a reduced mean arterial pressure sugges a dilatation of the resistance vessels under resting conditions (Bevegård and Shepherd 196 Beiser *et al* 1969) In this study similar results were found during both control and LBN conditions.

In conclusion, the results suggest that both a reduction in cardiac output and a decrea in peripheral vascular resistance may contribute to the carotid sinus induced reduction blood pressure but the relative importance of the two effector mechanisms depends on t hemodynamic situation. The hemodynamic adjustments induced by LBNP result in a increase in blood pressure but a decrease in the heart rate regulating capacity of the carot sinus.

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Methods

male rats of Sprague-Dawley strain bred at this Institute and weighing about 350 g at the start of the experiment were used. Under ether anesthesia one of the following three surgical procedures was performed: section of the hypogastric nerve on both sides distal to the hypogastric ganglia 1 cm below the bifurcation of the aorta; section of the pelvic nerves close to the urinary bladder and extirpation of the pelvic ganglion on both sides; and a combination of these two procedures. After the two latter types of operation, the bladders of the rats had to be emptied daily by manual pressure using ether anesthesia. To avoid bacterial infection the animals were given sulfonamide (Solfano[a] Nordmark Werke), 20 mg daily subcutaneously. 3 or 8 days after the operation the rats were either killed by inhalation of ether and the bladders removed for choline acetyltransferase assay or they were anesthetized with chloralose (100 mg/kg) through a femoral cannula after induction with ether and used for experiments in which the hypogastric nerves were stimulated electrically.

Estimation of choline acetyltransferase

The bladders of 56 rats were analysed; unoperated litter mates served as controls. The method used was that devised by Catherine Hebb (see Nordenfelt 1963). Acetone dried powder of the bladders was prepared and then made up in cysteine-saline in a concentration of 25 mg/ml or, when the enzyme activity was expected to be low as in the case after extirpation of the pelvic ganglia alone or in combination with section of the hypogastric nerves, in a concentration of 50 mg/ml. Of the tissue extracts 0.2 ml were incubated at 38°C for 1 h. After the incubation had stopped the incubate volumes were adjusted to 40 ml of frog Ringer's solution or, when low enzyme activity was expected to 20 ml. The acetylcholine synthesized was then assayed on the eserinized frog rectus muscle. When gallamine ($4 \cdot 10^{-4}$ g/ml) was added to the bath the incubate caused no contraction of the muscle and when the incubate was boiled for a short time in alkali (but not in acid) its activity was destroyed, both findings, which seem to confirm that the active substance studied was acetylcholine.

The enzyme activity is expressed in μg acetylcholine chloride formed per h per urinary bladder (total activity) and in μg acetylcholine chloride formed per h per g acetone powder (concentration).

The incubation medium was such that the activity of rabbit brain, used as a reference sample, was 9 200 μg ACh/h/g acetone powder. Activities less than 10 μg ACh/h/g acetone powder could not be measured on the rectus muscle.

Stimulation experiments

18 rats were used. The bladder was exposed and the ureters were ligated. A glass cannula was inserted into the bladder through an incision in the urethra. The bladder was filled with 0.25 ml of physiological saline and the pressure development by the detrusor muscle was recorded by means of a transducer and polygraph. The resting pressure was about 5 mmHg in all experiments.

The hypogastric nerves were cut distal to the hypogastric ganglia and the distal ends were stimulated electrically at about the level of the bifurcation of the aorta, using bipolar electrode. A Grass stimulator, giving rectangular pulses with a duration of 2 ms, frequency of 20 or 100 Hz and of supramaximal intensity (10 V) was used.

Statistics

Student's t-test for paired samples was used; comparisons were made between the operated rat and its unoperated litter mate. The 0.05 level of probability was accepted as significant.

Drugs

The substances used in the stimulation experiments were: hexamethonium bromide, atropine sulphate, dihydroergotamine methanesulphonate and guanethidine bensulphate. The drugs were injected through the cannula in the femoral vein.

Results

Activity of choline acetyltransferase

The total enzyme activity of the normally innervated urinary bladders in the 20 rats studied, expressed in μg acetylcholine formed per h per bladder was 10.2 ± 0.7 (mean ± S.E.). The

Acta physiol. scand. 1977 101 58–62
From the Institute of Physiology University of Lund, Sweden

Choline Acetyltransferase Activity in the Denervated Urinary Bladder of the Rat

By

Jörgen Ekström and Mats Elmér

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Abstract

Ekström J and M Elmér *Choline acetyltransferase activity in the denervated urinary bladder of the rat* Acta physiol scand. 1977 *101* 58–62.

After extirpation of the pelvic ganglia the choline acetyltransferase activity decreased markedly indicating that most of the postganglionic cholinergic neurones of the bladder take this route. A small decrease in the activity of this enzyme was found after section of the hypogastric nerves, showing that these nerves contribute to some extent to the cholinergic innervation The residual enzyme activity found after a combination of the two surgical procedures suggests that neurones relay distal to the level of the section of the hypogastric nerves and pass outside the pelvic ganglia and (or) that neurones pass the pelvic ganglia and relay distal to them Electrical stimulation of the hypogastric nerves after extirpation of the pelvic ganglia and the use of blocking drugs showed o the existence of cholinergic neurones passing outside the pelvic ganglia, some of them relaying distal to the point of stimulation.

Key words: Urinary bladder choline acetyltransferase, denervation

Stimulation experiments have shown that the detrusor muscle of the rat receives cholinergic fibres not only from the pelvic but also from the hypogastric nerves (Elmér 1975 a). The number of acetylcholinesterase positive nerves in the bladder however does not seem to change by section of the hypogastric nerves, while no such nerves could be found after extirpation of the pelvic ganglia (Alm and Elmér 1975) the hypogastric nerves are considered to pass through the pelvic ganglia (Langworthy 1965) As to the acetylcholine content in the rat bladder about 10 per cent is still found after extirpation of both pelvic ganglia (Carpenter and Rubin 1967) However motor responses could not be elicited by transmural stimulation of bladders thus operated (Carpenter and Rand 1965)

In this connection it appeared to be of particular interest to study the effects of section of the hypogastric nerves or extirpation of the pelvic ganglia, or a combination of the two procedures on the activity of the acetylcholine forming enzyme, choline acetyltransferase, previously shown to be present in the rat bladder (Ekstrom 1975 a) The results obtained led to experiments in which the motor effects of electrical stimulation of the hypogastric nerves after extirpation of the pelvic ganglia were studied

Stimulation experiments

Electrical stimulation of the hypogastric nerves at 20 Hz 8 days after extirpation of the pelvic ganglia caused contraction of the detrusor muscle in 8 out of 10 rats. The intravesical pressure response was 1.7±0.4 mmHg (n = 8). After the injection of hexamethonium, 10 mg/kg, the contraction of the bladder was reduced in 2 out of 4 rats, the reduction being about 50%. Atropine, 1 mg/kg, reduced the response by about 60% in all the rats, while dihydroergotamine, 2 mg/kg, had no effect (Fig. 1).

Stimulation of the hypogastric nerves at 100 Hz gave a response in all the rats and increased the intravesical pressure by 2.0±0.4 mmHg (n = 10), which was significantly higher than the response to stimulation at 20 Hz ($p < 0.05$). The increased part of the response caused by the high stimulation frequency was not affected by previous injection of hexamethonium, 10 mg/kg, or atropine, 1 mg/kg, but was totally abolished by guanethidine, 1 mg/kg, or dihydroergotamine, 2 mg/kg (Fig. 1).

Discussion

The profound fall in the total activity of the choline acetyltransferase in the bladder to about 1 per cent or less of the normal value after bilateral extirpation of the pelvic ganglion, shows that most of the cholinergic postganglionic neurones reach the urinary bladder via this pathway. The decrease in total enzyme activity observed at an early stage after severance of the hypogastric nerves shows that these nerves contribute to the choline acetyltransferase activity of the bladder and thus, supply the bladder with cholinergic neurones. The finding of a residual enzyme activity when extirpation of the pelvic ganglia was combined with section of the hypogastric nerves suggests the presence of cholinergic neurones relaying distal to the level where the hypogastric nerves were sectioned and passing outside the pelvic ganglia and (or) of cholinergic neurones passing the pelvic ganglia and relaying distal to them. The possibility that some fibres of the hypogastric nerves actually pass outside the pelvic ganglia was confirmed by the increase in intravesical pressure when the hypogastric nerves were stimulated after the pelvic ganglia had been extirpated in advance. That the hypogastric fibres stimulated at physiological frequency were cholinergic is suggested by the outcome of the experiments where atropine and dihydroergotamine were used. From this type of experiment, using the ganglion blocking substance hexamethonium, it appears also that ganglion cells may occur distal to the stimulation point. It should be pointed out that the partial resistance to atropine observed is a well known finding (see Elmér 1975 b, Carpenter 1977). High stimulation frequencies are known to stimulate the adrenergic fibres of the hypogastric nerves activating excitatory -adrenoceptors in the rat bladder (Elmér 1975 c). By the use of such a high frequency in the present study it is shown that also adrenergic fibres reach the bladder without passing the pelvic ganglia, however they apparently do not relay below the level of stimulation. Concerning the possible presence of ganglion cells distal to the anatomically distinct pelvic ganglion Alm and Elmér (1975) stimulated the nerve close to the bladder but the effects did not appear to be blocked by hexamethonium. A small number of intramural ganglion cells, thought to be cholinergic, has been found in some morphological studies on the rat bladder (El-Badawi and Schenk 1966, Chesher 1967).

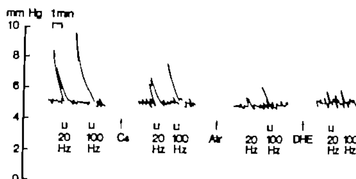


Fig. 1 Pressure responses of a urinary bladder to electrical stimulation of the hypogastric ner[...] extirpation of the pelvic ganglia C6 hexamethonium, 10 mg/kg, Atr atropine, 1 mg/kg, DHE [...] ergotamine, 2 mg/kg.

concentration of the enzyme activity in μg acetylcholine formed per h per g acetone [...] was 837±56 The bladders weighed 73 1±1.9 mg.

The enzyme activity was markedly lower in the bladders of the rats from which the [...] ganglion had been extirpated bilaterally 8 days previously than in the normal co[...] In 2 of the 8 bladders examined it was, in fact, below the limit of detection (less tha[...] and 0.31 / g/h/bladder) In the remaining 6 the total enzyme activity and the concen[...] (per g acetone powder) were 0 51±0 07 and 15±2.2 μg ACh, respectively The bl[...] after this type of operation were found to be 3–4 times heavier than those supplied [...] normal innervation

In the 10 rats in which the hypogastric nerve had been sectioned bilaterally 8 da[...] viously the total enzyme activity of the bladders was almost significantly higher (p [...] than that of the controls, being 9 6±1.3 and 7 8±1 0 μg ACh, respectively In te[...] concentration (per g acetone powder) the activity was 850±102 and 691±72 μg A[...] difference which is significant at the $p<0\ 01$ level The weights of the bladders of the [...] groups did not differ from each other

In contrast to the above mentioned finding at 8 days urinary bladders of 10 rats exa[...] for their choline acetyltransferase activity 3 days after the section of the hypogastric [...] had a total activity which was 16% lower ($p<0\ 01$) than in the 10 control rats, being 10.[...] and 12.5±0 5 μg ACh, respectively The concentration (per g acetone powder) of the en[...] activity in these operated rats was 884±46 and in the control rats 982±59 μg ACh [...] difference tended to be significant ($0\ 05<p<0\ 1$) When examined at this early sta[...] bladders of the operated rats were found to be 9% lighter ($p<0\ 05$) than those of [...] respective controls.

Extirpation of the pelvic ganglia combined with bilateral section of the hypogastric [...] did not further reduce the very low acetylcholine forming capacity seen after the rem[...] of the ganglia alone. Thus 8 days after the combined surgical procedure 1 out of [...] bladders studied had an enzyme activity which was not detectable (less than 0.33 μg A[...] h/bladder) while the total enzyme activity in the others was 0 82±0.17 μg ACh In term[...] concentration (per g acetone powder) the activity was 23±4 8 μg ACh. The bladders sho[...] a similar weight increase as that seen after extirpation of the pelvic ganglia alone.

Acta physiol. scand. 1977 101 63–71

From the Brain Research Laboratory E-blocket, University Hospital, and the Department of Neurosurgery University of Lund, Sweden

Adenosine in Rat Cerebral Cortex. Its Determination, Normal Values, and Correlation to AMP and Cyclic AMP during Shortlasting Ischemia

By

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Abstract

Nordström, C. H., S. Rehncrona, B. K. Siesjö and E. Westerberg. *Adenosine in rat cerebral cortex. Its determination, normal values, and correlation to AMP and cyclic AMP during shortlasting ischemia*. Acta physiol. scand. 1977 *101* 63–71

[illegible] has recently been suggested that adenosine is [illegible] metabolic coupling factor responsible for an increased cerebral blood flow during hypoxia or increased functional activity. However, tissue adenosine concentrations have been reported to increase in situations previously shown to be unassociated with changes in tissue AMP concentrations. The present experiments were undertaken to assess cerebral cortex concentrations of adenosine under normal circumstances, and to relate changes in adenosine, AMP and cyclic AMP during shortlasting ischemia. Following freezing and extraction of tissue, adenosine was measured using high pressure liquid chromatography. In paralyzed and anesthetized (70% N_2O) rats, freezing of tissue through intact skull bone gave an adenosine concentration of 0.9±0.1 μmol kg^{-1} (mean±S.E.M.). With freezing through the exposed dura the concentration was 3 times as high with [illegible] large scatter. When special precautions were taken to avoid tissue trauma during craniotomy the adenosine concentration was 1.1±[illegible]1 μmol kg^{-1}. It is concluded that previously reported values are erroneously high. During the first 60 [illegible] of total ischemia there [illegible] a better correlation between increase in AMP and in adenosine concentration [illegible] [illegible]ll as between adenosine and cyclic AMP concentrations). It is concluded that increases in tissue adenosine concentration only occur if AMP accumulates. However, since (relative) changes in adenosine concentrations are at least twice those of AMP, analyses of adenosine may provide sensitive measures of change in phosphorylation state.

Current interest in adenosine in the brain centers on two aspects of its metabolism, related to regulation of neuronal function and cerebral blood flow respectively. *First*, adenosine is postulated to activate [illegible] denylate cyclase and thereby to cause increased formation of cyclic AMP (Sattin and Rall 1970, McIlwain 1972). Second, when locally applied to cerebral [illegible] rterials adenosine has been shown to be a potent vasodilator (Berne *et al.* 1974, Wahl and Kuschinsky 1976). As a result of these and other observations it has been suggested that adenosine acts as one of the regulators of cerebral blood flow during physiological conditions (Rubio and Berne 1975, Rubio *et al.* 1975).

In other studies no ganglia could be found in the bladder wall of this species (Carpente Rubin 1967 Alm and Elmér 1975)

The observation that the choline acetyltransferase activity was not decreased, but h tended to be increased, in the urinary bladder after cutting the hypogastric nerves t previously was surprising. This phenomenon may be due to an increase of the activity c enzyme in the cholinergic nerves present and (or) to an extension of these nerves in the der both possibilities that could be explained by an increased nervous activity as a res to the loss of one of the nervous pathways. Other experiments mainly on salivary g indicate that the activity of choline acetyltransferase is dependent on the traffic of impulses (see Ekström 1975 b 1977 a, b) It should be pointed out, that the effect o enzyme activity is probably not a consequence of the degeneration of the adrenergic neu of the hypogastric nerves, since treatment with 6-hydroxydopamine causing a sel destruction of neurones of this type is not followed by any change in the choline a transferase activity of the urinary bladder of the rat (Ekström 1975 a).

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NORDENFELT I Choline acetylase in normal and denervated salivary glands. *Quart J xp Ph siol.* 48 67 79

concentration due to slight tissue trauma, the results do not lend themselves to temporal analysis of changes in adenosine concentration during ischemia. However the results allowed correlation between the concentrations of adenosine, AMP and cyclic AMP.

All brains were classified out during intermittent irrigation with liquid nitrogen and stored at $-80°C$ until extraction.

Analytical methods. The cerebral cortex below the craniotomy and the corresponding area in the non-operated animals were dissected at $-22°C$ in refrigerated glove box. The samples were weighed and extracted with HCl-methanol. The tubes were then brought to 0°C, perchloric acid was added and the extraction was completed as previously described (Folbergrová *et al.* 1972). Phosphocreatine (PCr), ATP, ADP, AMP, lactate and pyruvate were measured with enzymatic fluorometric methods (Lowry and Passonneau 1972, see Folbergrová *et al.* 1972). Cyclic AMP was determined according to Gilman (1970), as described elsewhere (Folbergrová *et al.* 1977).

Analyses of adenosine were performed with high pressure liquid chromatography (Waters, model U6K universal Injector, 6000 A Solvent Delivery System, 440 UV Absorbance Detector). A nonpolar column (μ Bondapak C 18) was used as the stationary phase and mixture of 90 ammonium phosphate buffer and 10 methanol, pH 7.1 as the mobile phase (reversed phase operating mode). The absorbance was measured at 254 nm. One hundred μl of the neutralized brain extract was injected directly into the column. Adenosine showed retention time of about 12 min (see below), with an eluent flow of 1.0 ml min^{-1}. The detector sensitivity was 0.0025 AUFS (Absorbance Units Full Scale). Adenosine was identified by comparison of absorbance maxima for variety of nucleotides, nucleosides and bases (see Results).

Calculations. The energy charge of the adenine nucleotide pool (E.C.) was calculated according to Atkinson (1968) as

$$E.C. = \frac{[ATP] \quad 0.5\,[ATP]}{[ATP] \quad [ADP]+[AMP]}$$

Statistical differences were evaluated with the student's t-test and linear regression analyses according to conventional methods.

Materials. μ Bondapak®-C 18 column (27324) was obtained from Waters Associates, Inc., Gothenburg, Sweden. Methanol Pronalys was obtained from May & Baker Ltd. Dagenham, England, and ammonium phosphate for buffer solutions from Merck, Darmstadt, West Germany. Adenosine was supplied by Boehringer Mannheim, West Germany. Cyclic AMP assay kit (TRK. 432) from The Radiochemical Centre, Amersham, England, was used for c-AMP analyses.

Results

Measurements of adenosine concentration

The Waters model 440 UV detector is designed for a recorder input of 10 mV giving a maximal sensitivity of 0.005 AUFS. The sensitivity was increased to 0.0025 AUFS by reducing the input potential to 5 mV. A further reduction of input potential resulted in a disturbing base line noise.

Fig. 1 gives the retention time for a number of nucleotides, nucleosides and bases. As can be seen, adenosine had retention time of 12.4 min, while AMP and its other degradation products gave quite different retention times.

The adenosine concentrations were quantitated from peak height measurements and calculated from standard runs. Each day 3 to 5 samples of known amounts of adenosine were analysed prior to measurements of tissue extracts. In each instance, adenosine was dissolved in the extraction blank used for the fluorometric analyses. Fig. 2 shows that there was a linear correlation between adenosine concentration in standard samples and peak height.

It has been shown by *in vitro* experiments that adenosine is released from cerebr upon electrical stimulation, and following hypoxia or hypoglycemia (Pull and M 1972, 1973) Corresponding *in vivo* studies indicate that electrical stimulation or cause increases in tissue adenosine concentration (Rubio *et al* 1975) Adenosine is from AMP in a reaction catalyzed by 5'-nucleotidase (E.C. 3 1.3 5). The rate of this appears to be determined by substrate availability *i.e* by the AMP concentration *et al* 1972, see also Pull and McIlwain 1973) This raises the question whether o increased tissue concentration of adenosine can be expected always to be present in s accompanied by pronounced vasodilation, *e.g* hypoxia and epileptic seizures. Thus, data demonstrate that even relatively marked hypoxia is unaccompanied by a de increase in AMP concentration (Bachelard *et al* 1974 Norberg and Siesjö 1975), epileptic seizures in paralyzed and well oxygenated animals lead to a very small accum of AMP if any (Chapman *et al* 1977) There are two possible explanations for the d results (1) previous *in vivo* data on adenosine concentration are influenced by changes due to inadequate tissue fixation, or (2) adenosine is a more sensitive indica change in phosphorylation state than AMP

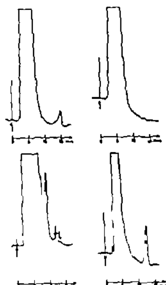
The present study was undertaken to estimate cerebral cortical adenosine concen using *in situ* freezing techniques that cause minimal autolytic changes, and to changes in adenosine AMP and cyclic AMP in ischemia of maximally 60 s dur will be shown that normal adenosine concentrations are considerably lower than pr reported and that there is a strict correlation between changes in adenosine and shortlasting ischemia.

Experimental procedures

Preparation of tissue Male Wistar rats (275–350 g), with free access to water and rat pellets until were used i the experiments. Anaesthesia was induced with 2% halothane to allow tracheoto animals were then immobilized with tubocurarine chloride and ventilated with 70% N_2O and to keep arterial P_{O_2} bove 100 mmHg and arterial P_{CO_2} between 35 and 40 mmHg. One femoral a cannulated for continuous recording of the blood pressure and anaerobic sampling of arterial bloo analyses. The body temperature was kept close t 37°C. I some of the animals, parietal craniot performed (see below). Following operation, all nimals were allowed a steady stat period of at leas

Control levels of metabolites were btained from three groups of animals. In *group A* a skin inc made and the skull bone exposed to accommodate a plastic funnel for *in sit* freezing of the br liquid nitrogen (Pontén *et al.* 1973). It has been shown that, with this freezing technique, the cerebr underlying the exposed skull bone freezes in 10–20 s however since unfrozen parts of the tissue tinuously supplied with well oxygenated blood, utolytic changes seem to be avoided (Pontén *et a* I *group B* a parietal craniotomy (8 × 8 mm) was performed on the left side, exposing the dura the steady state period (see bove) the dura was kept moist with prewarmed, isotonic NaCl-soluti brains from these animals were frozen by superfusi g the dura with Isopenthane® precooled to its point (−156°C), allowing freezing of the superficial 1 mm of the cerebral cortex in about 1 (Nilss 1975). On analyses, it was found that there was pronounced variability in adenosine concentratic since corresponding variability was observed in other labile metabolites as well (ADP AMP nd it was concluded that operative trauma was involved. Theref re, new series (*group C*) was colle four different operators who took special preca tions not to traumatize the tissue. These precaut cluded the use of a low-speed burr which was pplied with intermittent cooli g of the bone by m irrigation with a NaCl-solution, and the use of operating microscope for dislocating the bone fr

I group B, separate animals were decapitated, nd the tissue was frozen with isopenthane after 30, 40, and 60 s of ischemia. Since some of these brains must have had a preischemic increase in ad



3. Four chromatograms (for conditions see Fig. 1) showing: extraction blank, stored for several hours at room temperature, disturbing peaks at 13 and 14.7 mm can be seen. B, fresh extraction blank, here the disturbing peaks are almost eliminated. brain tissue sample from one of the control animals, with disturbing peak (↑) in relation to adenosine peak (↑). D Adenosine in standard solution prepared in the extraction blank.

ndard addition technique which should reduce the error to about +2%. It should be lded that the errors calculated above represent upper estimates since they are based on und area quantitation. The errors should be smaller when band peak height quantitation is ed

Adenosine concentration in control brains

able I gives the values for mean arterial blood pressure and body temperature, as well as the arterial P_{CO_2}, P_{O_2} and pH, in the three groups studied (see Methods). These physiological variables were very similar between the groups, excluding the possibility of an effect on cerebral metabolism.

Labile cerebral metabolites are given in Table II. In group A, i.e. in animals whose brains were frozen through the intact skull bone, concentrations of PCr ATP ADP and AMP were similar to those previously reported from the laboratory (e.g. Norberg and Siesjö 975), while cyclic AMP concentrations are similar to those given in a recent report (Fol-

TABLE I. Physiological parameters in the three control groups (group A, brain tissue frozen through intact skull bone, groups B and C through exposed dura). Values are given as means ± S.E.M.

	Group A (n 10)	Group B (n 6)	Group C (11)
Mean arterial blood pressure, mm Hg	160 ± 4	140 ± 4	150 ± 3
Temp °C	37.2 ± 0.1	37.3 ± 0.2	37.4 ± 0.1
$PaCO_2$, mm Hg	40.0 ± 1.0	37.6 ± 0.7	38.9 ± 0.7
PaO_2, mm Hg	124 ± 5	133 ± 5	123 ± 3
pH	7.393 ± 0.012	7.370 ± 0.014	7.388 ± 0.006



Fig. 1 Retention data for a number of nucleotides, nucleosides and b
(Adenosine at 12.4 min.)

Conditions:
Column 1/4 1 μ Bondpak C18
Solvent: 90% $(NH_4)_2$ HPO } pH 7.1
10% MeOH
Sample 100 μl
Flow rate 1.0 ml/min
Detector UV 254 nm, 0.0025 AUFS.

Two problems were encountered in the analysis of adenosine. First, if the extract solution was stored at temperatures above −20°C, two peaks (Fig. 3 A) with retention ti very close to that of adenosine developed (at 13 and 14.7 min). Tests with exclusion of ed EDTA or imidazole from the solution indicated that the peaks were due to oxidation p ducts of these substances. In fresh extraction solutions, these peaks were almost undetecta (Fig. 3 B), the peak at 14.7 min being about one third of that due to adenosine in extra from control brains (Fig. 3 C). The error due to the latter peak can be estimated to ab +3% (Snyder and Kirkland 1974). The peak at 13 min, which was indistinguishable fr that due to adenosine, explains why the line of Fig. 2 did not pass through the origo. avoid interference by this peak the standard solutions were prepared in extraction bla of equal age as the brain sample extracts (see Fig. 3 D). Second, some of the brain samp were analysed for other metabolites before adenosine was measured. In these samples t disturbing peak at 14.7 min had reached a height about twice that due to adenosine, giv an estimated error of about −10%. To avoid this error, these samples were analysed wit

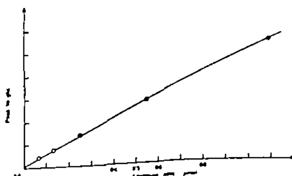
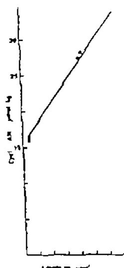


Fig. 2. Correlation between peak height a adenosine concentration in 5 standard so tions. (For conditions see Fig. 1.)



5 Relation between tissue concentrations of adenosine (μmol kg⁻¹) cyclic AMP (μmol kg⁻¹) in the ischemic animals (16) and the pooled trol values (square represents mean ± S.E.M from 28 control animals) equation of the regression line: Cyclic AMP (μmol kg⁻¹) = 0.15 adeno- (μmol kg⁻¹) + 1.52 (0.84 p > 0.001).

e data show a linear relationship between AMP and adenosine concentrations with a high gree of correlation (r 0.99 p 0.001). The correlation shows that an increase in AMP ncentration of 0.017 μmol g^{-1} was associated with a doubling of adenosine concentration rom 1 to 2 μmol kg^{-1}).

Fig. 5 gives the relationship between tissue concentrations of adenosine and cyclic AMP the ischemic animals (16 animals) and the pooled control values (28 animals). There was linear relationship between adenosine and cyclic AMP concentrations up to adenosine ncentrations of about 10–15 μmol kg^{-1} Experiments with longer periods of ischemia ot shown) indicated that although there was a marked further increase in adenosine con- ntration the cyclic AMP concentration did not increase above 3.5 μmol kg^{-}

Discussion

The present results are pertinent to questions regarding the normal tissue concentration of denosine, and relationship between adenosine, AMP and cyclic AMP These questions will e discussed in turn.

Tissue adenosine concentrations in control situations

It is well known that, in brain tissue, degradation of AMP occurs by deamination to IMP and phosphohydrolysis to adenosine, catalyzed by AMP-deaminase (E.C. 3.5.4.6) and 5′-nucleotidase (E.C 3.1.3.5), respectively (.g Deuticke *et al* 1966, McIlwain and Bachelard 1971). The further metabolism of IMP and adenosine leads to formation of inosine in re- actions catalyzed by 5′-nucleotidase and adenosine deaminase (E.C. 3.5.4.4), respectively In brain tissues, a perturbation of the phosphorylation state with an increase in AMP con- centration leads to increases in adenosine, IMP and inosine; however results obtained on

TABLE II Cerebral cortex concentrations of labile metabolites in animals whose brains were frozen through the intact skull bone (group A) or through the exposed dura (groups B and C). In group C special precautions were taken to avoid trauma to cerebral cortex. Values are means ± S.E.M. in μmol g⁻ except for adenosine and cyclic AMP that are given in μmol kg⁻. In group A and C lactate and pyruvate concentrations were measured in only 4 and 12 animals, respectively

	Group A (n = 10)	Group B (n = 6)	Group C (n = 18)
PCr	4.56 ± 0.06	3.75 ± 0.11	4.12 ± 0.07
ATP	2.96 ± 0.02	2.82 ± 0.02	2.89 ± 0.03
ADP	0.284 ± 0.003	0.333 ± 0.014	0.277 ± 0.005
AMP	0.043 ± 0.004	0.056 ± 0.009	0.044 ± 0.001
E.C.	0.944 ± 0.001	0.931 ± 0.004	0.943 ± 0.001
Lactate	1.58 ± 0.06	2.51 ± 0.42	1.73 ± 0.09
Pyruvate	0.116 ± 0.008	0.123 ± 0.013	0.123 ± 0.006
Lac/Pyr	13.7 ± 0.7	20.8 ± 2.8	14.1 ± 0.5
Adenosine	0.9 ± 0.1	2.9 ± 1.0	1.1 ± 0.1
Cyclic AMP	1.58 ± 0.09	1.73 ± 0.08	1.62 ± 0.05

$= p < 0.05$ $= p < 0.01$ and $= p < 0.001$ E.C. = adenylate energy charge.

bergrová *et al* 1977) The adenosine concentrations were one third to one fifth of those reported by Rubio *et al* (1975) in the same species.

In group B comprising brains frozen through the exposed dura, the mean adenosine concentration was almost three times as high, with a large scatter (0.6–6.7 μmol kg⁻). A corresponding variability was observed for ADP (0.284–0.382 μmol g⁻), AMP (0.039–0.097 μmol g⁻), lactate (1.6–4.2 μmol g⁻) lactate/pyruvate ratio (11.0–31.5), and adenylate energy charge (0.917–0.941) Since it was suspected that this variability was caused by tissue trauma in part of the material, the experiments were repeated with special precautions to avoid trauma (see Methods) The results of this series (group C) corroborated the suspicion since values for labile phosphates were now similar to those obtained in group A Most importantly the adenosine concentration was close to 1 μmol kg⁻

Correlation between adenosine AMP and cyclic AMP in short-lasting ischemia

The relationship between AMP and adenosine concentrations, as obtained in 17 animals subjected to complete cerebral ischemia from 10 to 60 s, is given in Fig. 4 The control values (28 animals) from groups A and C were pooled and are given as a single point (open circle).

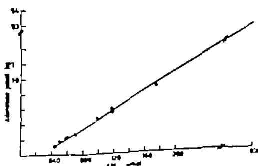


Fig. 4 Relation between individual values of adenosine (μmol kg⁻) and AMP (μmol g⁻) in 17 animals subjected to complete cerebral ischemia from 10 to 60 sec. The control values are given as a single point (open circle) representing mean from 28 animals (groups A and C). The regression line, thus representing individual data from 45 animals, is given by the equation adenosine (μmol kg⁻¹) = 0.06 AMP (μmol kg⁻¹) − 1.7 (r = 0.99 p < 0.001).

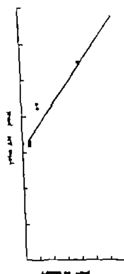


Fig. 5 Relation between tissue concentrations of adenosine (μmol kg⁻¹) and cyclic AMP (μmol kg⁻¹) in the ischemic animals (16) and the pooled control values (square represents mean ± S.E.M. from 28 control animals). The equation of the regression line: Cyclic AMP (μmol kg⁻¹) 0.15 adenosine (μmol kg⁻¹) 1.52 (r = 0.84, p 0.001).

The data show a linear relationship between AMP and adenosine concentrations with a high degree of correlation (0.99 $p < 0.001$). The correlation shows that an increase in AMP concentration of 0.017 μmol g^{-1} was associated with a doubling of adenosine concentration (from 1 to 2 μmol kg^{-1}).

Fig. 5 gives the relationship between tissue concentrations of adenosine and cyclic AMP in the ischemic animals (16 animals) and the pooled control values (28 animals). There was a linear relationship between adenosine and cyclic AMP concentrations up to adenosine concentrations of about 10–15 μmol kg^{-1} Experiments with longer periods of ischemia (not shown) indicated that although there was marked further increase in adenosine concentration the cyclic AMP concentration did not increase above 3.5 μmol kg^{-1}

Discussion

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rat, rabbit and cat brains indicate that the pathway via adenosine is the most active (Deuticke *et al* 1966, Matthias and Busch 1969 Kleihues *et al* 1974). Recent results sug that the rate of production of adenosine in the 5 -nucleotidase reaction is mainly determi by substrate supply (Kluge *et al* 1972) *Thus it can be expected that whenever there is* increase in AMP concentration the adenosine concentration will rise and, since the c centration of adenosine is less than 5% of that of AMP it may be a sensitive indicator change of phosphorylation state.

In view of what has been discussed above it seems clear that true *in vivo* concentration adenosine can only be obtained if the fixation technique gives minimal autolytic artefa Freezing through the intact skull bone has been shown to give optimal concentrations phosphocreatine and adenine nucleotides (Pontén *et al* 1973). However since freezing the tissue is slow there is the possibility of changes in labile metabolites due to differen effects of hypothermia on reactions leading to production and further metabolism of dividual metabolites (see results reported on phosphorylase by Folbergrová *et al* 1977).

Freezing through the exposed dura avoids a prolonged period of hypothermia (Nil *et al* 1975) However as the present results show craniotomy easily leads to minor th damage, and to changes in the concentrations of some labile metabolites. Only if spe precautions are taken to avoid tissue damage is it possible to obtain values comparable those measured after freezing through the intact skull bone. The results then obtai demonstrate that the *in vivo* concentration of adenosine is close to 1 μmol kg

There are few previous measurements of tissue adenosine concentrations, and in so studies the methods have not been well suited to measure concentrations of the order 1 μmol kg⁻ (Deuticke *et al* 1966, Matthias and Busch 1969 Kleihues *et al* 1974). In experiments of Rubio *et al* (1975), a craniotomy was performed and the brain was fro using small bone rongeurs precooled in liquid nitrogen. This probably explains why cont *values were in the range 3 to 5 μmol kg⁻ (cf results on group B, Table II)*. This conclus is corroborated by the high values for lactate concentration and lactate/pyruvate ra reported by these authors (see also values for AMP in Fig. 6 in Rubio *et al* 1975)

Relationship between adenosine AMP and cyclic AMP concentrations

The present results corroborate the results of Rubio *et al* (1975) in showing a linear corre tion between increases in AMP and adenosine concentrations. The combined results are line with the conclusion of Kluge *et al* (1972) that the rate of adenosine production in 5 -nucleotidase reaction is determined by the concentration of AMP In other words, th are no reasons to assume that adenosine should accumulate in the absence of an increase AMP concentration. However since a 50 % increase in AMP is associated with a doubl of adenosine concentration it is possible that increases in extracellular adenosine co centrations of physiological importance can occur in situations, *e.g* hypoxia, in wh changes in AMP concentrations escape detection (see Introduction). This problem can o be studied by means of analyses of tissue concentrations of adenosine, using adequate *in s* freezing techniques. Comparable problems arise in interpreting the correlation betwe increases in adenosine and cyclic AMP concentrations.

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Capillary Permeability to Albumin in Normotensive and Spontaneously Hypertensive Rats

By

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Received 14 March 1977

Abstract

RIPPE, B and B FOLKOW *Capillary permeability to albumin in normotensive and spontaneously hypertensive rats* Acta physiol. scand. 1977 *101* 72-83

Transcapillary passage of plasma proteins is enhanced in man's primary hypertension and it is debated whether this reflects increased permeability or merely a raised capillary pressure. To elucidate this problem maximally vasodilated hindquarters of spontaneously hypertensive rats (SHR) and normotensive control rats (NCR) were perfused in parallel at constant flow with dextran, horse serum or mixtures of the two, with labelled albumin as indicator of capillary permeability to macromolecules. By equal increases of venous pressure modest filtration was maintained during one hour after which the edema and its albumin content were determined. — There was less edema in SHR reflecting a slightly lower postcapillary resistance and a much higher precapillary resistance compared with NCR, which here resulted in a lower capillary pressure in SHR. In both SHR and NCR the presence of dextran slightly enhanced the capillary filtration coefficient but increased albumin permeability up to tenfold, also after antihistamine drugs. However for each perfusate the SHR capillaries were, if anything, slightly less permeable to albumin than the NCR ones. — The results suggest that the enhanced transcapillary passage of plasma proteins in primary hypertension reflects an increased capillary pressure in some circuit(s), probably mainly skeletal muscle, resulting from the functional balance *in* / between the pre and postcapillary resistances.

Quantitative studies concerning design and principal hemodynamic characteristics of the different series-coupled vascular sections have been sparse in primary hypertension, particularly when compared to the situation in normotension However such a study was recently performed by Folkow *et al* (1974) in spontaneously hypertensive rats (SHR Okamoto 1969), in comparison to normotensive control rats (NCR). The results showed that the structural increase in wall/lumen ratio generally characterizing large arteries and resistance vessels in primary as well as secondary hypertension (*cf* Folkow *et al* 1973), is largely confined to the *precapillary* vessels, at least in the hindquarter section of the systemic circulation. Thus, the capillary section in the preparation used seemed to be essentially unchanged in SHR, insofar as the "isogravimetric" capillary pressure and also the capillary filtration coefficient (CFC) values were the same as in NCR at maximal dilatation. The postcapillary resistance vessels appeared to be, if anything, structurally slightly wider in the SHR than in the NCR hindquarter vascular bed and they exhibited no sign of any "hyperreacti ity

constrictor or dilator agents, a response which characterizes the precapillary vessels (Folkow et al. 1974). Such observations indicate that the increased precapillary resistance in hypertension "protects" the exchange and postcapillary venous vessels from the impact of the increased arterial pressure, though the situation may later change if e.g. a failing heart leads to an increased central venous pressure which is transmitted in the retrograde direction.

It was, however, recently discussed by Parving (1975) whether capillary permeability might be increased already in early phases of essential hypertension in man, in respects that are not necessarily reflected in CFC measurements. This proposal was mainly based on the fact that already in early essential hypertension there occurs an increased transcapillary escape rate (TER) of intravenously injected labelled albumin (cf. Parving and Gyntelberg 1973, Ulrych 1973). Capillary permeability is, however, only one of the factors determining the rate of transcapillary exchange of plasma proteins, another important factor being the level of capillary pressure. Thus, there is good evidence that elevations of capillary hydrostatic pressure increase the net protein escape across the exchange vessels (e.g. Landis et al. 1932, Szabo, Magyar and Papp 1963, Haddy, Scott and Grega 1972, Larsen, Parving and Rossing 1974).

For such reasons the increase of TER, observed in subjects with essential hypertension, may simply be due to a raised capillary pressure. Ulrych (1973) has, for example, discussed whether an increased TER might ensue as a result of vasoconstriction particularly involving the venous side. However, it is in this connection of considerable relevance that muscle blood flow is commonly enhanced in essential hypertension (cf. Pickering 1968), particularly in its early "hyperkinetic" phases (cf. Julius and Shork 1971) simulating the hemodynamics of very light exercise or, perhaps better, in a mild defence reaction (cf. Folkow and Neil 1971, Julius and Esler 1975). In such situations the precapillary resistance of the muscle circuit is lowered compared with most other circuits, while venous capacitance tone is, if anything, increased. Particularly when associated with an increased arterial pressure, this must imply a substantial enhancement of capillary pressure in skeletal muscle tissue. Inevitably the Starling equilibrium in this large tissue mass is then shifted towards net filtration and with this follows an increased capillary transfer of plasma proteins, as during normal exercise. A quite recent study (Rippe, Kamiya and Folkow 1977) provides strong evidence against the often proposed view that macromolecules should mainly pass capillary walls by way of micropinocytosis in such situations.

In experiments on man it is very difficult to distinguish clearly between e.g. a slight deviation in the capillary permeability-surface characteristics (PS product) and one in mean capillary pressure, particularly when comparing mild, uncomplicated cases of essential hypertension with normotensive controls. Therefore, if primary hypertension really involves a deviation in capillary PS characteristics, the chances of revealing such a change would be better if animal models of primary hypertension are utilized instead. Since this problem is one of principal interest in the evaluation of hypertension in general, it was considered worthwhile to perform a quantitative comparison between SHR and NCR hindquarters with regard to their capillary permeability to proteins under strictly controlled experimental conditions. This was done, keeping the entire capillary bed open to flow while maintaining other hemodynamic factors as equal and constant as possible during paired SHR-NCR

perfusions with plasma and different plasma substitutes, containing labelled album tracer of transcapillary protein transfer

Methods

Technically successful experiments were performed on ?9 pairs of Wistar spontaneously hyperten (SHR Okamoto 1969) and weight matched Wistar normotensive control rats (NCR). All rats ere sex and weighed between 290–410 g. The majority of them were 6–9 months old and hence in n early r medi m phases of established hypertension. The isolated hindquarters of one SHR weight matched NCR were simultaneously perfused t constant rate and at essentially equal flows outflow pressures were moderately elevated but to an *equal* extent in the two animals, to induce a capillary filtration and consequent transfer of plasma proteins.

Preparation. Initially mea arterial blood pressure (P_A) was measured in the awake experimental *via* a polyethylene can ula, inserted into the tail artery during brief ether anesthesia, utilized for recordings throughout the experiment *via* Statham P23 AC transducer writing on a Grass Pol Afterwards the animals were anesthetized with Nembutal 3 mg/100 g administered into the ta can ula, simultaneously eviscerated and the abdominal aorta and caval vein were freed bet ee th and iliolumbar vessels. The hindquarters were otherwise completely isolated by standardized mass at identical levels just proximal to the iliolumbar vessels, and the tail distal to the tail artery cannula paws were excluded so that the hindquarter preparation mainly consisted of skeletal muscle (*cf* *et al.* 1970).

After heparinization the bdominal aortas in both rats were ligated, immediately cannulated in th direction and connected to a common perfusion system which was already run g. The two cav were then ligated, immediately can ulated and connected t 30 cm lo g outflow tubes, after b animals were killed. The pressure drop along the two venous outflow tubes was equal and bout 5 at a perfusion rate f 10 ml/min. Their outflow tips could be raised or lowered about 25 cm and to that venous o tflow pressures of the two preparations were always identical the tips were linked to

To induce moderate filtration, venous outflow pressures (P) were elevated t an identical exten two preparations, usually from 6 up to 18 mmHg. In some of the paired experiments venous pr just proximal to the o tflow tubes were measured directly *via* cann lae in venous side branches and to be identical at given levels of outflow pressure. The artificial perfusio system utilized as c t both animals, as described in detail earlier (*.g* Folkow *et l.* 1970).

Perf sates. It i known since long that perfusio of vascular beds with g dextran affects capillar brane characteristics, considered t be mainly due t the bsence of native proteins (*cf* Landis and F helmer 1963 Areekul 1969). To investigate the problem further in these comparisons of norm and hypertensive vascular beds, 4 different perfusates were employed, *iz* horse serum (Normal SBL, Sweden), dextran (Macrodex®-70· MV bout 70.000 generously supplied by AB Pharmacia, S and two different mixtures of dextra nd horse serum. All colloids were mixed with Tyrode soluti the perfusates were kept t 38°C and ygenated Because of the differences in perfusat the anima divided int 4 groups.

In *group I* si matched pairs of NCR SHR were perfused with horse serum (containing 65 g of per liter), diluted with Tyrode t 60 per cent of ts initial concentration, *i* a 3 9 per cent content of p

I *group II* eight matched pairs of NCR SHR were perfused with 4% dextran in Tyrode.

In *group III* eight matched pairs of NCR SHR were perfused with dextra in Tyrode of the sam centration as in group II but with addition f 5 g/l of horse serum protein.

In *group IV* seven matched pairs of NCR SHR were perfused with mixture of dextran–Tyro g/l) and native horse serum, in the proportions of 2 1 The final dextran concentration was the and the protein concentration bout 2%.

Control" stage f perfusion The vascular beds were itially "flushed" for 2 3 min by adjust pump to deliver 15–20 ml/min 100 g tissue in order to wash out ll traces of blood. T induce m dilatation, repeated doses of papa erine, 1 2 mg t a time, were given t both hindquarters *ia* a co mixing chamber in the perfusion system ntil no further pressure drop occurred This stat of m dilatation was kept stable throughout by repeated papaverine i jections or papaverine infusion.

During the subsequent 10–15 min flow was kept low t 3–5 ml/min 100 g tissue, wh le venous o pressure was near t zero, as indicated by the readily visible collapse of both enous trees. The hindq capillary bed is then slightly bsorbing, as observed earlier (Folkow *t al.* 1974) nd confirmed in e

... here the hindquarters were continuously weighed. This was done to avoid initial edema formation that the preparations would preferably have slightly reduced interstitial fluid space. At the end of this period, flow were increased identically and varied rapidly during few minutes in order to obtain data constructing flow-pressure curve for each preparation, which better characterizes the dimensions their resistance vessels. Some filtration was thereby induced, more or less restoring the initial fluid balance, as shown from experiments here the hindquarters are continuously weighed.

Perfusion with tracer. After these initial stages of perfusion, tracer quantities (1 · 10^{-3} g/l) of ^{125}I-Human Serum Albumin (Radiochemical Center, Amersham, England; γ-radiation energy 0.035 MeV) are added to the perfusate while the two venous outflow cannulae were raised identically to reduce filtration. Flow was then kept around 9 ml/min · 100 tissue and equal in the two preparations. This period of perfusion with addition of labelled protein to the perfusate was continued for 60 min, the venous effluents being collected and, after aeration and reoxygenation, recirculated once or twice. Arterial pressure was continuously checked and it was ensured that maximal vasodilatation was throughout maintained in both preparations. Thus, the entire capillary surface area was exposed to flow throughout and at moderately raised pressure levels because of the increased venous outflow pressure.

The specific activity of the perfusate was determined repeatedly (see below) and its radioactivity fell about 2–3 % in 1 h because of the recirculations. Non-protein bound radioactivity of the perfusate was determined before and after the perfusion period as specific activity of the supernatant, following precipitation of the perfusate proteins with 10 % trichloroacetic acid. It varied from 1.0–1.8 per cent of total perfusate radioactivity but did not change in the course of a given experiment.

After about 60 min of perfusion with the tracer-containing perfusate, a rapid shift to perfusion with tracer-free, but in other respects identical perfusate was performed. This tracer-free perfusion was stopped when the specific activity of the venous effluent was well below 3 per cent of the initial activity, which usually occurred after 7–8 min. During this final tracer-free perfusion period, the venous outflow cannulas were lowered to the level of the animals in order to minimize further filtration.

Determination of tracer transfer. After stopping the tracer-free perfusion, three separate muscle groups from each of the two hindquarters were dissected out bilaterally, namely 1) the anterior crural group (m. tibialis ant., m. extensor dig. longus and m. extensor hallucis proprius); 2) m. gastrocnemius and soleus, 3) the larger part of m. quadriceps. Immediately after dissection each muscle preparation was put into a test tube. After sealing and weighing, the tube was put into a well counter, connected to a Packard Auto-gamma Spectrometer (Model 410 A), for determination of radioactivity by counting a minimum of 10 000 imp. Each muscle was then completely dried at 90–100°C for 72 h and reweighed.

Wet weights (W_c) and dry weights (D) of corresponding muscle groups from 6 NCR and 6 SHR, not used for experiments, were determined separately as controls. The SHR and NCR muscles did not differ mutually concerning the relationship of dry weight/wet weight. The following normal values for the various muscle groups, expressed as per cent of their wet weights ($(D/W_c) \cdot 100$), were determined and used as controls: 1) the anterior crural muscle group 24.7 per cent, 2) m. gastrocnemius-soleus 24.2 per cent, 3) m. quadriceps 23.6 per cent. Knowing these percentages ($(D/W_c) \cdot 100$) for muscles from non-perfused "control" animals, together with the dry weights and wet weights after perfusion (W_1) from the muscles of the experimental groups, the edema content ($W_1 - W_c$) of the "experimental" muscles, expressed in g per 100 g of tissue, could be determined. Muscle radioactivity corrected for background activity was then divided by the edema content ($W_1 - W_c$) and by the specific activity of the perfusion medium. Correction for free iodine activity was not deemed necessary because the free iodine is supposed to be distributed extracellularly and should thus easily be washed out during the 8 min of final tracerfree perfusion.

In this way the ratio between the albumin concentration of the newly formed "edema fluid" (C_T) and that of the perfusate ("plasma") (C_P) was obtained for every muscle group and animal. All the experiments, especially those of group I, were performed under conditions of moderately enhanced capillary filtration. The purpose was to create a situation in which the transcapillary albumin flux was largely filtration dependent, while absorption and net diffusion could be neglected (concerning micropinocytosis (see Rippe, Kamiya and Folkow 1977). Furthermore, escape by means of lymphatic flow could also be ignored since practically all lymphatic vessels were blocked by ligation during preparation. The newly formed edema must then be largely equal to the filtrate formed during the experimental period.

Under these conditions the filtrate/plasma concentration ratio of albumin, C_T/C_P, will approach A_a/A_w, i.e. the ratio of "effective" pore area available to albumin to that of water. The same deduction can be made from the relation developed by Pappenheimer (1953) $C_T/C_P = (PS \cdot FA_a/A_w)/(PS + F)$, where C_T/C_P is the filtrate/plasma concentration ratio and PS the diffusion capacity for the macro-molecule, while F is the filtration rate. At high filtration rates C_T/C_P will be a good approximation of A_a/A_w, and thus fairly

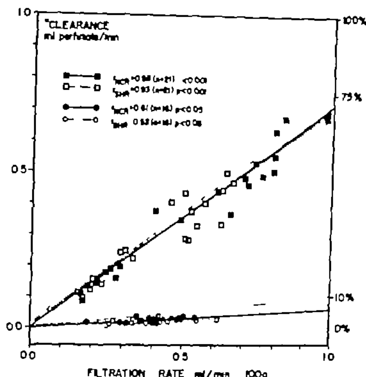


Fig. 1 Relationship between perfusate clearance of albumin and filtration rate for NCR and SHR groups I (circles 60% horse serum and 40% Tyrode) and II (squares 4% dextran in Tyrode). As seen dextran perfusion implies a tenfold increase in albumin clearance but without any appreciable difference NCR SHR. Further for each of the perfusates shown in the Fig. C_F/C_P remains essentially constant independent of the filtration rates used, which was also the case for the other two perfusates (groups III and IV). — Each point represents one out of three duplicate determinations per animal, performed on three different muscle groups which varied somewhat concerning extent of edema formation.

constant. In the present experiments C_F/C_P could thus be used as a measure of capillary permeability to albumin. At the prevailing filtration rates there was namely a good correlation between the "plasma" clearance of albumin and filtration rate in all groups, i.e. almost constant C_F/C_P ($\approx A_F/A_P$) was obtained.

Statistical evaluation was performed by the pairing design t test mean values of the six determinations of C_F/C_P in every SHR being compared with the corresponding NCR values. To obtain comparison between the differently perfused groups, the groups comparison t-test was utilized. At p values below 0.05 the differences were considered significant.

Results

A Filtrate to "plasma" albumin concentration ratios (C_F/C_P)

1 *C_F/C_P at different filtration rates and different "plasma" compositions.* A good correlation was found between the rate of edema formation and net transcapillary outflux of albumin in all animal groups. Independent of the filtration rate (which varied between 0.1 1.5 ml/min 100 g depending on the venous pressure level as related to the respective levels of isogravimetric capillary pressure for the different perfusates) the concentration of protein in the filtrate remained almost constant for a given perfusate. This was evident by the fact that the concentration of tracer albumin in the filtrate vs that in the perfusate did not change appreciably with increasing filtration and this was so for both NCR and SHR (Fig.

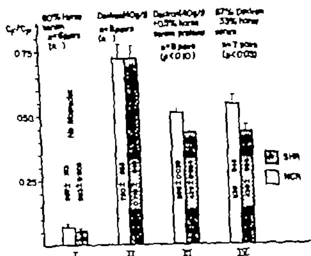


Fig. 2. Filtrate to perfusate concentration ratios of labelled albumin (C_F/C_P) in NCR and SHR during perfusion with diluted horse serum (group I) or dextran-containing perfusates (groups II–IV, see text). Each observation on a given animal represents the mean value from three duplicate determinations, for every paired group of experimental animals means ± S.E. are given in the figure. — Note that C_F/C increases markedly, however the perfusate contains dextran but that the SHR capillaries are, if anything, less permeable to protein than the NCR ones for each perfusate used.

During perfusion with horse serum-Tyrode (group I) the percentage of tracer protein in filtrate versus "plasma" was as low as 5–7% in both NCR and SHR (see also Fig. 2). When 40 g/l of dextran (Macrodex®) in Tyrode was employed as perfusate (group II), C_F increased tenfold, being 0.70–0.75 in both NCR SHR, but if small amounts of horse serum proteins (5 g/l) were added to this dextran perfusate (group III), C_F/C decreased to 0.45–0.5. However as long as dextran was present no further reduction of C_F/C_P was seen when the protein concentration was increased up to 20 g/l (group IV).

C_F/C in SHR compared with NCR. Mean values of C_F/C in the six SHR hindquarters perfused with horse serum (group I) was 0.053 ±0.008 compared with 0.067 ±0.013 in the matched NCR (Fig. 2), values which closely agree with findings in blood perfused cat muscle (Appelgren, Jacobsson and Kjellmer 1966). Although NCR here showed a somewhat higher C_F/C_P than SHR in 5 expts. out of 6, the difference was not statistically significant. Anyway it seems safe to conclude that SHR does not show any higher albumin permeability than NCR. The results are in agreement with earlier findings concerning identical capillary filtration coefficients in SHR and NCR hindquarters at maximal dilatation (Folkow et al. 1974), confirmed in other more recent measurements (unpublished).

During perfusion with dextran alone (group II) C_F/C increased, as mentioned, about tenfold in both NCR and SHR, and this was so also in preparations given large amounts of an antihistaminic agent. However under these conditions of a very marked increase in capillary membrane permeability to albumin, C_F/C_P was identical in SHR and NCR being 0.7 ± 0.05 (mean ±S.E.) in both. After addition of protein to the dextran perfusate, first 5 g/l (group III) and then 20 g/l (group IV) mean C_F/C values were reduced to 0.51 ±0.04 in NCR and to 0.43 ±0.03 in SHR (15 pairs) with no significant differences between groups

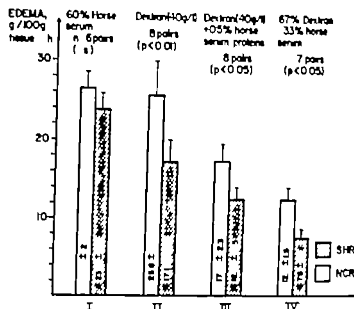


Fig. 3 Amount of edema (mean ± S.E.) formed during one hour of perfusion ith the four perfusates. Although capillary pressure was kept largely equal in all groups, 17 18 mmHg in NCR effective filtr pressure varied between the four groups due to d fferences in colloid osmotic pressure further CFC differed modestly with the perfusate (see text). In NCR effective filtration pressure as estimated to 14 mmHg in group I and some 9 7 and 5 mmHg, respectively in groups II III IV the corresponding va being 1 2 mm lower in SHR.

III and IV However here the slightly lower values in SHR differ significantly from those in NCR (Fig. 2)

B Edema formation

1 *Differences in edema formation rate in the various groups.* The edema formation during 1 h of tracer perfusion varied significantly between the 4 groups (Fig. 3). The explanation is partly that effective filtration pressure, *i.e* the difference between the prevailing mean hydrostatic pressure in the capillaries (Pc) and the isogravimetric one (Pci), differed between the groups depending on the perfusates used.

In NCR mean Pc was estimated to 17 7 ± 0 4 mmHg during the period of raised P in the majority of experiments, as calculated from the prevailing P_A and P_V values and from the pre- to postcapillary resistance ratio (which at these distending pressures is about 3 in NCR according to Fig. 1 in the paper by Folkow *et al* 1974) This Pc value during the filtration period did not differ significantly in the various groups of NCR as tested with variance analysis. The same calculations for SHR in the various groups (where the P increases were always identical to the paired NCR and where the prevailing pre- to post capillary resistance ratio is rather about 5 1 according to Folkow *et al* 1974) resulted in a mean value for Pc of 16.6 ± 0.4 mmHg at the prevailing P_A and P_V levels. This Pc level in SHR was significantly lower than that in NCR (p < 0 001), and the results by Folkow *et al* 1974 as well as unpublished results with the isogravimetric technique are, in agreement with a true difference in Pc between NCR and SHR when P_V and flow are equal

From these Pc values the effective filtration pressures can be approximately estimated for the four groups of NCR and SHR To start with group II (4 dextr n) the Pci valu

round 9 mmHg for both NCR and SHR, as judged from unpublished "isogravimetric" stimations using the dextran perfusate. This gives an effective filtration pressure of about mmHg in NCR with the SHR value being 1 or 2 mmHg lower which at least in part explains the different edema amounts (Fig. 3).

In group III (4% dextran + 0.5% horse serum proteins) the edema formation was some 30–35 lower than in group II. This is likely to reflect the moderately higher colloid osmotic pressure of this perfusate, in association with some reduction in capillary permeability as caused by the protein addition, and independent CFC measurements show this. However the CFC reduction is only of the order of 20–25%, where the raised colloid osmotic pressure is likely to account for the rest of the difference between groups II and III. A proper fit with the present data is obtained if the 0.5 protein addition raised Pcl from 9 to about 11 mmHg. If so the effective filtration pressures in group III would amount to about 7 mmHg in NCR and 6 mmHg in SHR (Fig. 3).

In group IV (2.7% dextran + 2% horse serum proteins) the edema formation again decreased significantly presumably due to a further increase in colloid osmotic pressure of the perfusate. 4% dextran exerts colloid osmotic pressure in the skeletal muscle vascular bed only some 16 mmHg (Eliassen *et al.* 1974), and 2.7 per cent dextran mixed with 2% serum proteins, which per unit weight exerts about the same colloid osmotic pressure as dextran, must imply a colloid osmotic pressure that is perhaps 2.5–3 mmHg higher than for 4% dextran. Consequently the effective filtration pressures would here be in the order of 4–5 mmHg in NCR and 2–3 mmHg in SHR.

Finally in group I (3.9 horse serum proteins) the colloid osmotic pressure is bound to be a few mmHg below that in group II, being about 14–15 mmHg and resulting in a Pcl around 8 mmHg for both NCR and SHR. The *effective* filtration pressure in NCR would then be 10–11 mmHg and 1–2 mmHg lower in SHR, *i.e.* somewhat higher than for group II. However CFC is lower (20–25) for horse serum than for dextran, as mentioned above, so the rate of edema formation would be expected to be fairly equal in groups I and II as also shown in Fig. 3. — These estimations of differences in net filtration pressures with the various perfusates are admittedly approximate but are likely to be realistic as to their general order. They are necessary to explain the differences in edema formation between the four groups of experiments and also for the comparison between SHR and NCR.

2. *Amount of edema in SHR and NCR.* Although the paired SHR-NCR hindquarters were always exposed to identical venous pressures, the SHR hindquarters showed throughout lower amount of edema formation than NCR during the 60 min of perfusion, as shown in Fig. 3. Though this difference was quite small and not significant in group I, it was more substantial in the dextran group I (p 0.01) and in groups III and IV ($p < 0.05$) where dextran and horse serum were mixed. These differences can hardly be ascribed to any differences in CFC, since SHR and NCR here show equal values (Folkow *et al.* 1974 unpublished observations). They rather suggest first a lower mean hydrostatic pressure in SHR than in NCR as result of differences in both pre- and postcapillary resistances (*cf* Folkow *et al.* 1974). Second, there might be a slightly smaller leakage of macromolecules to the interstitial space in SHR (see Fig.) which, if anything, would maintain a somewhat higher effective colloid osmotic pressure in the SHR capillaries. In any case, there are

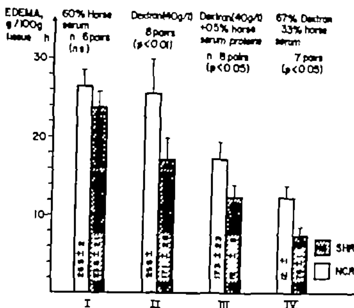


Fig. 3 Amount of edema (mean ± S.E.) formed during one hour of perfusion with the four perfusates … Although capillary pressure was kept largely equal in all groups, 17 18 mmHg in NCR, effective filtra… pressure varied between the four groups due to differences i colloid osmotic pressure further CFC … *differed modestly with the perfusate (see text). In NCR effective filtration pressure was estimated to* … mmHg in group I, and some 9 7 and 5 mmHg, respectively In groups II III, IV the corresponding val… bel g 1 2 mm lower in SHR.

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From these Pc values the effective filtration pressures can be approximately estimated fo… the four groups of NCR and SHR To start with group II (4 % dextran) the Pci value is hen…

—ypertensive crises with serious consequences particularly for the brain (e.g. Johansson 974). The precapillary autoregulatory response then yields to the overwhelming rise in all tension, thereby transmitting the greatly increased transmural pressure to the exchange essels. However the structural precapillary changes typical of hypertension here offer relative protection, since their stronger media does not yield as easily as that of normotensive essels (cf Hallbäck, Lundgren and Weiss 1974 Johansson 1975).

In any case, the present experiments on an animal model of man's essential hypertension rovides no evidence of either any "primary" or secondary" increases in capillary permeability in SHR, whether in terms of protein permeability or CFC (Folkow et al. 1974). t is then likely that the increased TER in essential hypertension (Ulrych 1973, Parving 1975) eflects a hemodynamic situation where instead mean capillary pressure is somewhat raised n at least some of the major systemic circuits. In fact, also young SHR show a tendency of n increased TER in the resting" awake steady state (Rippe, Lundin and Folkow 1977). As mentioned in Introduction, muscle blood flow is commonly increased in essential hypertension and particularly so in early "hyperkinetic stages (cf Julius and Shork 1974), when the hemodynamic pattern simulates that of a mild defence reaction or ery light exercise The muscle vasodilatation in these situations is mainly precapillary in nature, which inevitably implies a raised capillary pressure, and therefore an increased formation of filtrate. With this follows an increased transcapillary albumin escape, apparently mainly by means of increased filtration through large pores (bulk flow), while capillary permeability per se n all likelihood remains unchanged (cf Arturson and Kjellmer 1964). A concomitant presence of a raised central venous pressure due to g venoconstriction will, of course, further end to enhance the filtration by retrograde effect on the pressure level in the microvessels.

The present experiments further showed a less pronounced edema formation in SHR compared with NCR (Fig. 3), despite largely equal CFC values and isogravimetric capillary pressures (Folkow et al 1974). Filtrate to plasma concentration ratios of albumin (C_F/C) were in most experiments slightly reduced in SHR compared to NCR (about 15%). Therefore capillary permeability to macromolecules is, if anything, lower in SHR than in NCR. As result the total outflux of labelled albumin into the SHR hindquarter interstitial fluid space was up to some 35% lower in SHR. This indicates that effective filtration pressure must have been throughout lower in SHR than in NCR, in extent varying with the perfusates used as outlined in Fig. 3 and essentially resulting from a different balance between pre and postcapillary resistances in SHR, since venous outflow pressure and flow were equal. At maximal vasodilatation the SHR hindquarter vascular bed displays a substantially higher precapillary resistance than the NCR one (Folkow et al. 1974), but had this been the only difference at equal flows mean capillary pressure would have also been equal. Therefore, both the mentioned study and the present results suggest that postcapillary resistance of the hindquarter vascular bed is sl ghtly lower in SHR than in NCR during complete vascular relaxation. However in the in vivo situation, the superimposed neurogenic—myogenic smooth muscle tone may of course, readjust the hemodynamic situation over a wide range when necessary

The drastic increases in C_F/C caused by dextran and only moderately reversed by plasma proteins in the perfusate were in these experiments utilized for comparing the permeability

certainly no signs of any *increased* capillary permeability in SHR compared with N in the present results

Discussion

As outlined in Introduction, studies on man indicate an increased transcapillary exch rate of albumin (TER) in essential hypertension (Parving and Gyntelberg 1973 Ul 1973 Parving 1975) Whether this increased turnover rate is due to an altered capi permeability to macromolecules in general or simply caused by an increased mean capi pressure in some major systemic circuits, or to a combination of both, has not been analy in detail in hypertensive subjects Parving has discussed the former alternative, while Ul seems to be more inclined towards the latter view

It is of principal interest both concerning which type of early vascular changes that oc in primary hypertension and for the correct analysis of the hemodynamic situation in disorder to know whether also the capillary exchange vessels proper exhibit any e alterations in permeability It is, for example, theoretically possible that the geneti linked deviations in cardiovascular design and function might involve a "primary de tion also in this respect and/or that "secondary" changes may occur early in the de lopment.

Against such a background the present investigation was performed to study selecti albumin permeability and rate of edema formation during induced filtration in the artific perfused, isolated and maximally dilated hindquarter vascular beds of spontaneo hypertensive and normotensive control rats (SHR, NCR). The essential result was that th are no signs of any increased capillary permeability to albumin in SHR compared w NCR, nor do they differ in capillary filtration coefficient (*cf* Folkow *et al* 1974). If a thing, the SHR hindquarter vascular bed seems to show a slightly lower albumin permeabi than that of NCR. Thus, there is no evidence of any primary" increase of capillary p meability in this type of primary hypertension.

The capillary exchange vessels might as mentioned also display a secondary increase permeability e.g if they are in the course of hypertension frequently exposed to lo pressure rises. However such a possibility also seems to be excluded by the present findi in SHR, being in the phase of fairly well established" hypertension. The main reaso probably the fact that the increased resistance in established hypertension is largely confi to the *pre*capillary vessels (Folkow *et al* 1974), which tends to protect the capillaries fr pressure elevations except when the precapillary resistance for functional reasons is relativ reduced However even when this is the case, as normally occurs in the muscle circuit dur exercise or defence reactions, the capillary membranes prove to be quite resistant, at le as long as such pressure rises are within the "physiological" range. Perhaps the most dras example is the situation normally facing the lower limb capillaries of man while ere though precapillary autoregulatory responses, reflex adjustments and the venous "pum will also here protect the exchange vessels from undue pressure rises.

This by no means denies that acute, marked pressure increases may overcome the pr capillary counterregulatory mechanisms, causing vascular distension wall edema and al gross capillary leakage (Giese 1964 a, b), a situation evidently not seldom met with

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properties of the capillaries over a wide range of situations. The nature of these changes permeability is, however *per se* of interest and worthy of a brief comment already he though the problem will be dealt with in a subsequent study designed to compare sim taneously the filtration and diffusion events (*cf* Rippe and Stage 1976). The decrease capillary selectivity for macromolecules induced by dextrans has earlier been studied Areekul in the perfused rabbit ear At blood perfusion the reflection coefficient for t plasma colloids was 0.94 while it was only 0.3 at perfusion with 3% dextran concentratio (mol. w around 80 000) increasing to around 0 5 after adding 5 g/l of pig plasma protei to the perfusate. These values can be roughly transformed so as to correspond to the presen used sieve coefficients (C_F/C_P) by being subtracted from 1 and they are then closely simil to the C_F/C_P values of the present study (*cf* Fig. 2.) Further preliminary results on t isolated rat hindquarter preparation show that CFC increases some 35% by shifting fro horse serum perfusion to 4% dextran perfusion *i.e* from around 0 035 ml/mm 100 (*cf* Renkin and Zaun 1955) to around 0 048 ml/min 100 g at dextran perfusion at comple vascular relaxation. These changes in permeability to pore-bound water filtration are th small compared with the nearly tenfold changes in large molecular permeability (0 0 compared with 0 70)

On the basis of CFC determinations it has been claimed that the permeability change induced by dextran can be almost completely reversed by the addition of proteins to th perfusate (*cf* Landis and Pappenheimer 1963) In the present study the addition of ev small amounts of proteins to the dextran perfusate considerably reduced CFC but th albumin concentration in the filtrate remained fairly high even if substantial additions o plasma protein were given Thus, the presence of dextran molecules seems to more strong enhance capillary permeability to large molecules than that determining the filtration o water but we will, as mentioned come back to these problems in another study

The mechanisms behind these actions of dextran on the capillary membrane are no understood (*cf* Areekul 1969) but the influence on permeability to colloids shows some rela tionships to that exerted by *e g* histamin or bradykinin. Although dextran is known to relea histamin from mast cells in rats, infusion of even huge doses of the antihistaminic dru clemastin (Tavegyl® Sandoz) in four experiments did not affect the C_F/C_P values signifi cantly Further in rabbits histamin is evidently not released in response to dextran, and st the present data closely simulate those found in rabbits (Areekul 1969), implying that hista min is not likely to mediate the mentioned permeability changes induced by dextran. I might represent some type of a direct action of dextran on the pores, since there are reason to believe that micropinocytosis is not involved in these processes (Rippe, Kamiya an Folkow 1977).

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h cardiac and vascular muscles are dependent, to a great extent, on Ca influx from the ernal environment. In this respect, however smooth muscle effectors do vary consider y depending on their location along the vascular bed. For example, at Ca_o concentrations low that the portal vein is completely unresponsive to noradrenaline (NA), aortic strips l retain much of their NA responsiveness. Furthermore, the dependence on Ca_o seems o to vary with the type of muscle activation. Thus, upon graded reduction of Ca_o the ogenic activity of the portal vein vanishes well before its responses to NA administration ttler 1976), suggesting a more efficient mobilization of calcium stores when membrane polarization is induced by the adrenergic transmitter than when it occurs "spontaneously" Recent studies (Folkow *et al.* 1976, Sutter *et al.* 1977) on the responsiveness of the micro-scular sections, which are of particular hemodynamic importance because of their control pre- and postcapillary resistance, capillary flow distribution and of capacitance, indicate increasing dependence on external calcium the closer one comes to the capillary level. us, in this and in several other respects, the smooth muscle of the microvessels is more ated to that of the portal vein than to that of the aorta or pulmonary artery while the rger more proximal precapillary vessels appear to fall somewhere in between.

The question arises whether chronic hypertension, besides having hemodynamically portant effects on precapillary vessels design in terms of increased media thickness in sociation with a reduced internal radius (*cf.* Folkow *et al.* 1973 1974), may also involve anges of smooth muscle function in the microvessels in that the process of excitation-ntraction coupling is altered, as reflected, *e.g.* in a changed dependence on external lcium. In the past few years it has been a subject of much discussion as to whether a change f this coupling might be decisive for the altered reactivity of the resistance vessels (*e.g.* Bohr and Haeusler 1974).

For such reasons, an experimental analysis was performed of the extent to which the sistance responses are affected by Ca_o^{++} changes in normotensive Wistar rats (NCR) s compared with young and adult spontaneously hypertensive rats (SHR Okamoto strain) nd renal hypertensive rats (RHR, induced by unilateral renal clipping in NCR). Part of he results has been briefly reported in an earlier paper (Folkow *et al.* 1976).

Methods

I. Material

The present study as based on hemodynamic analyses of the hindquarter vascular bed, in most cases taking paired expts. to compare the resistance responses in hypertensive and matched normotensive animal rats

Three groups of paired animals ere utilized for this purpose 1) 10 young "prehypertensive" SHR, 1-week-olds (SHR), mean arterial pressure (MAP) being 111±1 mmHg as compared with their matched controls (NCR) here MAP as 98±1 mmHg. The pressures were measured directly in the cannulated tail artery under awake conditions. 2) 21 adult SHR, (SHR) with matched controls (NCR_A) SHR_A were 6-7 months of age and hence in the "established" phase of hypertension, ith an MAP of 150+4 mmHg, hile NCR had pressure of 111±3 mmHg. 3) 10 RHR, bout 3 months of age at the time of the hemodynamic analyses. 6 weeks earlier unilateral renal artery clipping had been performed and the subsequent rise in blood pressure as checked 3 weeks later by means of tail artery cannulation and direct MAP measurement. On the day of study MAP was 175±7 mmHg in these RHR as compared ith their matched controls (NCR_R) here MAP was 105 _2 mmHg.

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Dependence on External Calcium for the Noradrenaline Contractility of the Resistance Vessels in Spontaneously Hypertensive and Renal Hypertensive Rats, as Compared with Normotensive Controls

By

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Abstract

FOLKOW B M HALLBÄCK, J V JONES and M SUTTER. *Dependence on external calcium for the noradrenaline contractility of the resistance vessels in spontaneously hypertensive and renal hypertensive rats as compared with normotensive controls* Acta physiol scand 1977 *101* 84–97

Isolated hindquarters of rats were perfused at constant flow with a plasma substitute so that pressor responses to various concentrations of noradrenaline (NA) could be measured in consecutive sections of the vascular bed with normal (> 1.5 mM) or low (0.2 mM) Ca^{++} in the perfusate. The animals used were 6-week-old spontaneously hypertensive rats of the Okamoto strain (SHR_Y), normotensive controls of the same age (NCR_Y) 6-7-month-old SHR (SHR_A) and NCR (NCR_A) and rats made hypertensive by clipping of one renal artery (RHR) plus matched normotensive controls (NCR_R). Concentration-response curves to NA showed that constrictor responses to NA become more dependent on external calcium as one proceeds peripherally in the vascular system in both SHR_A and NCR_A with responses in proximal end being least and small pre-and postcapillary vessels being most dependent in both cases. In low calcium SHR_A retained their responses better than NCR_A whereas RHR retained their responses to NA poorer than did NCR_R. No significant differences in responses in low calcium were observed in SHR_Y compared to NCR_Y though a trend in the same direction as in SHR_A could be traced. It is concluded that there is no evidence that altered handling of calcium initiates vascular hyperreactivity in SHR but that the handling of vascular calcium in SHR differs from RHR and both differ from NCR.

The intracellular concentration of calcium ions is of key importance for the excitation-contraction coupling in muscle. However the various types of muscle differ considerably with regard to their intracellular stores of calcium which are utilized in the process of activation. Thus, while in skeletal muscle the amounts of intracellular calcium bound to the sarcoplasmatic reticulum are able to account for the total Ca^{++} release during excitation,

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Methods

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Three groups of paired animals were utilized for this purpose. 1) 10 young, "prehypertensive" SHR, 6-week-olds (SHR_Y), mean arterial pressure (MAP) being 111 ± 1 mmHg as compared with their matched controls (NCR_Y) .. here MAP .. as 98 ± 1 mmHg. The pressures were measured directly in the cannulated tail artery under awake conditions. 2) 21 adult SHR, (SHR_A) with matched controls (NCR_A). SHR_A .. ere 6-7 months of age and hence in the "established" phase of hypertension, .. ith an MAP of 150 ± 4 mmHg, .. hile NCR .. had .. pressure of 111 ± 3 mmHg. 3) 10 RHR, about 3 months of age at the time of the hemodynamic analyses. 6 weeks earlier .. unilateral renal artery clipping had been performed and the subsequent rise in blood pressure .. as checked 3 weeks later by means of tail artery cannulation and direct MAP measurement. On the day of study MAP was 175 ± 7 mmHg in these RHR as compared .. ith their matched controls (NCR_R) where MAP .. as 105 _ 2 mmHg.

II Preparation and measurements

R_T measurements Details of the preparation of the hindquarter vascular beds for paired perfusion, for comparisons of SHR_A–NCR_A SHR_Y–NCR_Y and RHR NCR_R have been described earlier in d (*e.g* Folkow *et al.* 1970 Folkow *et al.* 1974) and only a brief summary will be given below By ca placed mass ligatures the hindquarters of the two animals were completely isolated with the exception o aorta and the inferior caval vein. The tail and feet were excluded from the circulation, leaving a tissue about 75% of which is made up of skeletal muscle. Mean arterial inflow pressure (P_A) of each prepar was continuously measured by cannula inserted in the tail artery Both hindquarters were then perf in parallel with the same artificial plasma perfusate (4% Dextran in oxygenated Tyrode solution) at con and equal flows per min and per 100 g of tissue, with the caval vei widely opened so that P_V as vir zero In this type of paired experiment the changes i P_A reflect the changes in total resistance to (R_T) in the hindquarter vascular bed under constant flow conditions.

R_{AP} measurement To estimate possible differences between SHR and NCR with respect to the resist responses of proximal parts of the precapillary vessels or more distal pre- and postcapillary sectio 6 pairs of NCR_A and SHR_A pressure also was measured in a sidebranch to a superficial small a located on the lower calf (P) As flow was known and kept constant, $P_A - P$ reflects the resistance to offered by proximal parts of the pre-capillary resistance vessels (R_{AP}). Some of these measurements performed on animals where separate R_A and R_V estimations were also made (see below), others in where only R_T was estimated, but both preparations showed closely similar $P_A - P$ responses. Measurem of P were initially performed in the intact animals, before the hindquarter preparation was started. small artery and its cannulated side branch had an external diameter around 0.15–0.20 mm bei ma exposed. Prior t cannulation, it was first completely relaxed by local application of papaverine, but facilitate the insertion of the cannula and to give its maximal external diameter which was 0 2–0.3 at normal arterial pressure. Its vascular tone was soon regained and then the diameter was again red particularly when an increased vasoconstrictor fibre tone was initiated by graded bleeding. — I the s rats $P_A - P$ also reflects the relative contribution of more "proximal pre-capillary resistance vc (R_{AP}) to the total systemic resistance ($R_T = (P_A - P_V)/Q$) because flow is the same along all connec vascular sections and P_V can be assumed to be nearly zero.

R_A R_{AD} and R_V measurements. One gro p of SHR_A ($=6$) and NCR_A ($=9$) were used for sepa estimations f the precapillary and postcapillary vessel responses with technique which has earlier l extensively used for comparing SHR and NCR (*cf* Folkow *et al* 1974). Here the venous outflow pre (P_V) also was continuously measured in a side branch of the caval vein and P_V could be set at any l by means of an outflow tube inserted in the caval vein. Further the fully isolated hindquarter prepar was continuously weighed with great precision. Since the preparation is far more time-consuming technically demandi g, it was ot possible t do these particular experiments as paired perfusions of SI and NCR_A Instead, SHR_A and NCR_A perfusions were alternately used, great care being taken to per all preparat ry details and manoeuvres in exactly the same way

Initially the vascular bed was kept maximally dilated by injections of papaverine and P_A and P_V adjusted such that an isogravimetric state was established Then the capillary filtration coefficient (C was measured by transiently inducing equal and simultaneous increases of both P_A and P_V. This proce was f llowed by inducing selective increases, first of P_A and then of P_V, t such levels that equal de of filtration were nduced in the two situations. This allows f r an estimation of the mean capillary pre in the isogravimetric state (P_{CI}) (*cf* Elussen *et al.* 1974). Knowing P_A P_{CI}, P_V and flow it as pos t deduce separately the pre- and postcapillary resistances (R_A R_V, respectively) in the maximally di state.

R_A and R_V could also be estimated when steady stat levels of vasoconstriction had been induced step increase in the concentratio of NA in the infusio (see below), each increase superimposed on previous pressure level. Here P_C increase, resulting from the postcapillary vasoconstriction in the cons flow situation, was reflected by shift from the isogravimetric equilibrium t increasing degrees of filtrat With correction fo concomitant reductions in CFC, as estimated from the volume increase causes transient P_V increases, it could be deduced how much P_C had increased as result of the NA constrict Thus, from the actual levels of P_A P_C and P_V the respectiv increases in pre- and postcapillary resistan could, fo the purpose of compar.son, be deduced with considerable precision by procedures that have b used in several earlier analyses of R_A R_V P_C and CFC (g Folkow t al. 1974 Elussen *et al.* 1974).

The extent of total resistance responses ($R_T = R_A + R_V$) corresponded closely in these SHR_A NC used fo separate R_A and R_V responses, and in those where only R_T, or R_T together with R_{AP}, w

scored. I was then possible to deduce also the changes of resistance in the "distal" pre-capillary resistance sub (R_{AD}). Flow being known and constant, the changes of $P - P_C$ thus reflect the R_{AD} changes, just P P and P P reflect the R_{AP} and R changes, respectively.

III. Experimental protocol

all experiments here P was measured, the $P_A - P$ difference as first studied in the intact SHR$_A$ d NCR before and after temporary blood losses in order to follow the extent to which the resistance the proximal precapillary vessels (R_{AP}) is involved in neurogenic adjustments in relation to the overall stance. This as done to see whether the neurogenic R_{AP} responses in vivo and the in vitro responses to A corresponded as to their magnitude.

When the artifical perfusion was started, the hindquarter vascular beds were, in most cases, first perfused th the plasma substitute containing low Ca^{2}_{o} concentration (0.2 mMol). This was continued for at least ... to wash out local extracellular stores of Ca. At intervals the Ca^{2+} content of the venous effluent as measured and fell rapidly (within 5–10 min) to level that stabilized around 0.3–0.4 mMol. Evidently slow Ca influx into the blood stream as going on throughout the low Ca_o perfusion, presumably largely emanating from the considerable Ca_i stores in the large muscle mass and, to some extent, from the skeleton. Therefore, the immediate Ca_o environment of the vascular smooth muscle in the steady state conditions of low Ca^{2}_{o} perfusion may be expected to be closer to 0.3–0.4 mM than to 0.2 mM, but was certainly equal in the paired preparations since they were identically treated and showed the same venous Ca^{2}_{o} concentrations. *Early during this initial period of Ca^{2}_{o} wash-out, repeated papaverine injections were performed to secure an initial, fully relaxed state for the vascular beds.* It was during this state of complete relaxation that the manoeuvres for estimating P_{CI} and CFC were performed in the R_A R_V expts. on SHR and NCR.

The NA infusions were then administered into common mixing chamber so that both preparations always received equal NA concentrations and flows. When the vascular response to given NA concentration had reached steady state step-wise increase in NA infusion as performed, until the maximum obtainable vasoconstrictor (pressure) response had been achieved. This as tested by finally increasing the NA concentration until no further pressure changes could be induced in the constant flow condition. Usually 6–7 different NA concentrations were used in each experiment in order to provide satisfactory dose-response "resistance curve".

Next the NA infusion as stopped and the vessels allowed to relax fully. Subsequently the NA infusion was again started, usually at the lowest NA concentration that had earlier produced barely noticeable response. When this weak response was fully re-established, $CaCl_2$ infusion as added to the mixing chamber to give largely normal Ca^{2}_{o} concentration of the perfusate (1.5 mMol or above). As soon as the Ca ions reached the tissues there was an immediate and pronounced enhancement of the vasoconstrictor response. This as also the case when higher NA concentrations were tested in this way. The full range of NA concentrations and vasoconstrictor responses were repeated at this approximately normal Ca^{2+} concentration, up to the maximum obtainable constriction (pressure) levels.

Once this had been obtained, the NA infusion as again stopped and the vessels were allowed to fully relax once more. This as ensured by means of repeated papaverine injection. Usually the whole procedure, from the first NA infusion to the last at low and high Ca^{2+} levels, respectively took about 2 hr. In some experiments the resistance responses to NA were tested in the reverse order i starting at normal Ca^{2+}_{o} levels, but in principle the results were the same, particularly concerning the relationships between the normotensive and hypertensive animals.

Considerable oedema formation occurred only if the intense constrictions at the highest NA infusions at normal Ca_o were allowed to last for longer periods. The reason is that only then are more powerful postcapillary contractions induced which, because of the constant flow perfusion, proportionally increases P and hence causes more pronounced filtration. The modest rise in "tissue pressure" that in such situations could be induced towards the end of the experiment could, however be estimated after full vascular relaxation by stopping the inflow and measuring the modest rise in "residual" P_A level. This type of slight disturbance affected only the final few NA infusion episodes at normal Ca^{2} contraction and could, moreover easily be corrected for.

On the basis of the recorded changes in pressure drop along the vascular bed as whole, and along the consecutive vascular sections, dose–response "resistance curves" could be calculated and plotted, for both the entire vascular bed and for its respective consecutive resistance sections at "low" and at "normal" Ca concentrations. For statistical evaluation of the results the pairing design t-test was used in all expts.

where the paired perfusion technique was utilized When this was not the case as when R_A and $R_($ mations were performed in SHR_A and NCR_A the group comparison t-test was used. Differences considered to be significant at the P < 0.05 level

Results

I NA effects on total resistance (R_T) at high and low Ca_e^+

In paired, constant flow perfusions of the hindquarter vascular beds the relationship tween NA concentration and pressure (P_A) responses (reflecting directly the total resista R_T, responses since flow was constant) was studied at normal (1.5 2 mM) and low mM) Ca_e^{2+} concentrations in the following groups of animals 1) SHR_Y in comparison age and sexmatched NCR_Y 2) SHR_A and their matched NCR_A and 3) RHR (unila renal clipping during about 6 weeks) and their NCR_R The arrangement of paired perfus greatly facilitated the comparison between the normotensive and hypertensive vasc beds since perfusate composition flow temperature NA and Ca_e^{2+} concentrations then identical in both Consequently the ratio between the responses of the hyperten and normotensive vascular beds was quite consistent within each group of animals, variations between experiments were somewhat larger presumably due to accidental m differences in temperature concentrations, perfusate composition etc. Nevertheless, occasional exceptions, the spread within each group of paired animals was quite sm particularly considering the fact that the pressure (resistance) responses amplify the und lying changes in vascular smooth muscle length to about the fourth power accordin Poiseuille s law

As normotensive controls, Wistar rats from the same breeding colony were used in 3 groups, but since they were of different ages they differed moderately in mean arte pressure (MAP) being in NCR_Y 98 ± 1 mmHg, in NCR_R $105 \pm ?$ mmHg and in NC 111 ± 3 mmHg. This age-dependent difference in pressure was reflected also in the cu relating NA dose to resistance response ("resistance curves") at normal Ca_e^+ insofar the maximal pressor responses to NA (MPR) were 167 ± 11 mmHg for NCR_Y 188 mmHg for NCR_R and 213 ± 15 mmHg for NCR MPR estimating the maximal contrac strength of the media, largely reflects the media thickness in relation to the internal radi particularly when huge amounts of Ba are added to ensure that all contractile sites engaged (*cf* Folkow *et al* 1973 1974) However in this particular study only NA used but earlier experiences have revealed a fairly constant relationship between maxin NA and maximal Ba responses in NCR, as well as in SHR and RHR

A close relationship between MAP and MPR (and also resistance at maximal vasodilati and steepness of the resistance curves) was noted also when the hypertensive rats w compared with their respective controls at normal Ca_e In agreement with earlier findin (*cf* Folkow *et al* 1973 1974 Lundgren 1974) Thus, in SHR_T MAP was 111 ± 1 mml with a MPR of 207 ± 11 mmHg (25 % higher than in NCR_Y) In SHR_A these values we 150 ± 4 mmHg and 308 ± 14 mmHg (45 higher than in NCR_A) and in RHR 175 ± 7 mml and 265 ± 2 mmHg (40 higher than in NCR_R) The MPR relationships between the thr hypertensive groups and their normotensive controls are also schematically illustrated Fig. 1 A However as also observed earlier (Lundgren *et al* 1974), betwe

and MAP was considerably higher in SHR_A (308/150 = 2.05) than in RHR (265/175 = 1.52), indicative of a relatively more pronounced media thickening in SHR_A, as is usually also the case with left ventricular hypertrophy in SHR and RHR, when related to their respective MAP. Concerning other characteristics of the resistance curves such as the NA threshold, ED_{50} etc., the three NCR groups were closely similar and so also were the hypertensive groups, again in agreement with earlier observations. In such resistance curves ED_{50} denotes 50 of the maximum reduction in average *internal* radius of the respective resistance vessels.

At low Ca_o the various NCR groups also showed a close correspondence concerning the NA resistance curves and their maximal pressor responses. However even though the ratio between the normotensive and hypertensive resistance curves was also quite consistent within each group, a considerable discrepancy was seen at low Ca_o between SHR and NCR, between RHR and NCR and particularly when comparing SHR with RHR. This is illustrated in Fig. 1 where the effects of normal and low Ca_o^{++} on the R responses to NA are compared in the three paired groups of animals. Section A of this Figure illustrates the maximal R_T responses at normal Ca_o when the NCR value is standardized to 100% in order to more clearly show the relative deviations from the normotensive controls of the maximal R responses in SHR SHR and RHR. The responses at *low* Ca_o are also indicated in section A by the hatched areas and are expressed as per cent of the respective maxima at normal Ca_o.

In section B of Fig. 1 the maximal NA responses at *low* Ca_o are again shown for NCR, RHR, SHR and SHR. To facilitate a direct comparison between them, all the responses at normal Ca_o are set to 100% so that the deviations of the SHR SHR_A and RHR responses at low Ca_o^{++} from those of NCR are more readily seen.

It is clear from Fig. 1 that both the RHR and SHR responses at low Ca_o deviate substantially from those of NCR, and in opposite directions. The differences from NCR are highly significant for both RHR and SHR_A ($p<0.01$), but not for SHR though SHR_Y show the same tendency as SHR_A, i.e. better maintained NA responses at low Ca_o than in NCR. In other words, the resistance vessels of the renal hypertensive rats and the spontaneously hypertensive rats behaved quite differently at low Ca_o the former showing a less pronounced NA response than the normotensive controls, the latter a more pronounced one. The difference between RHR and SHR is, of course, even more significant ($p<0.001$).

II. NA effects on consecutive resistance sections (R_{AP}, R_{AD}, R)

R_{AP}. As already indicated in an earlier study (Sutter *et al.* 1977), the "proximal" precapillary vessels, as defined also in Methods, appear to contribute towards 25% of the total resistance (R) upon intensified sympathetic discharge in rats. This was judged from the increased P − P difference in relation to P P during stepwise increases of blood loss. The same is true for SHR, though the *absolute* R_{AP} increase was here greater and in proportion to the greater P P ($-R_T$) difference in SHR. During "resting" equilibrium *in vivo* on the other hand, R_{AP} contributes only some 10% to R in both NCR and SHR. This indicates that in both the normotensive and hypertensive rats the larger proximal resistance vessels are relatively more involved in the remote neurogenic control of

where the paired perfusion technique was utilized. When this was not the case, as when R_A ... mations were performed in SHR_A and NCR_A the group comparison *t* test was used. Differ... considered to be significant at the $P < 0.05$ level

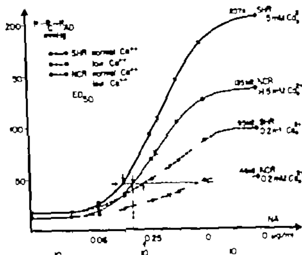
Results

I NA effects on total resistance (R_T) at high and low Ca_o

In paired constant flow perfusions of the hindquarter vascular beds the relation... tween NA concentration and pressure (P_A) responses (reflecting directly the total re... R_T, responses since flow was constant) was studied at normal (1.5–2 mM) and l... mM) Ca_o^+ concentrations in the following groups of animals 1) SHR_T in compar... age- and sexmatched NCR_T 2) *SHR_A and their matched NCR_A and 3) RHR* (m... renal clipping during about 6 weeks) and their NCR_R The arrangement of paired pe... *greatly facilitated the comparison between the normotensive and hypertensive* ... beds since perfusate composition, flow temperature, NA and Ca_o^+ concentratio... then identical in both Consequently the ratio between the responses of the hype... and normotensive vascular beds was quite consistent within each group of animal... variations between experiments were somewhat larger presumably due to accidenta... differences in temperature, concentrations, perfusate composition, etc. Neverthele... occasional exceptions, the spread within each group of paired animals was quite... particularly considering the fact that the pressure (resistance) responses amplify the... lying changes in vascular smooth muscle length to about the fourth power accor... Poiseuille s law

As normotensive controls, Wistar rats from the same breeding colony were use... 3 groups, but since they were of different ages they differed moderately in mean ... pressure (MAP), being in NCR_T 98 ± 1 mmHg, in NCR_R 105 ± 2 mmHg and in ... 111 ± 3 mmHg. This age-dependent difference in pressure was reflected also in the ... relating NA dose to resistance response ("resistance curves") at normal Ca_o^+ Ins... *the maximal pressor responses to NA (MPR) were 167 ± 11 mmHg for NCR_T* 1... *mmHg for NCR_R and 213 ± 15 mmHg for NCR. MPR, estimating the maximal con... strength of the media, largely reflects the media thickness in relation to the internal* ... particularly when huge amounts of Ba are added to ensure that all contractile si... engaged (*cf* Folkow *et al* 1973 1974) *However in this particular study only* N... used, but earlier experiences have revealed a fairly constant relationship between m... NA and maximal Ba responses *in NCR*, as well as in *SHR* and *RHR*

A close relationship between MAP and MPR (and also resistance at maximal vasodi... and steepness of the resistance curves) was noted also *when the hypertensive rats* ... compared with their respective controls at normal Ca_o In agreement with earlier fi... (*cf* Folkow *et al* 1973 1974 Lundgren 1974). Thus, in *SHR_T MAP was 111 ± 1* m... with a MPR of 207 ± 11 mmHg (25 higher than in NCR_T) in SHR_A these values ... 150 ± 4 mmHg and 308 ± 14 *mmHg (45 % higher than in NCR_A) and in RHR 175 ± 7* m... and 265 ± 22 mmHg (40% higher than in NCR_R). The MPR relationships between the ... hypertensive groups and their *normotensive controls are also schematically illustrat*... Fig. 1 A However as also observed earlier (Lundgren *et al* 1974) the *ratio* between

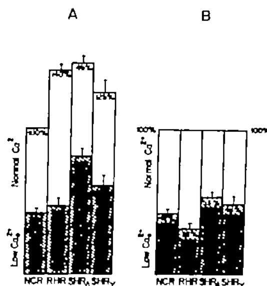


3 Dose-response R_{AD} curves to NA for SHR_A and NCR hindquarter vascular beds at normal and Ca_o^{++} during constant flow perfusion. The arrows indicate points in the curves where pressor responses are equal in magnitude and therefore allow comparison of the NA concentrations required to produce identical constrictions of the distal, precapillary resistance vessels at normal and low Ca_o in SHR_A and NCR_A. ED_{50} here denotes the point where 50 % of the maximum reduction in average internal radius of the distal resistance vessels is reached.

the R_{AP} response remains in both NCR and SHR. Both ways of expressing the responses can be justified and, in other words, the proximal resistance vessels behave quite similarly in both groups of animals.

R_{AD}. The maximal NA responses of the distal precapillary resistance vessels in SHR_A and NCR are shown in the middle part of Fig. 2, which also illustrates the extent to which these responses are reduced at low Ca_o. Since this vascular compartment, corresponding to the small distal arterioles and metarterioles, provides the dominating contribution to R_T and is, moreover, primarily responsible for the important myogenic tone and for local adjustments of flow and its microvascular distribution, the complete curves relating NA doses to pressor (resistance) responses for the R_{AD} section are also illustrated, both at normal and at low Ca_o (Fig. 3). As mentioned in Methods, these R_{AD} "resistance curves" were computed by subtracting R_{AP} and R from R even though all the various measurements could for technical reasons only exceptionally be performed in the same experiment. However there was such a close correlation with regard to the magnitude of the R_T responses to NA in expts. where either P_A alone, P and P_a, or P_A, P_a, P_O and P were estimated, particularly regarding percentage differences in SHR_A/NCR relationships, that the results could be pooled. Furthermore, the R_{AP} and R responses differed little between expts. and constituted such relatively small fractions of R during vasoconstriction, that possible errors in measurement will have little effect only on the computation of R_{AD}.

Figures and 3 illustrate how the R_{AD} responses were normally much steeper in SHR_A and also better maintained at low Ca_o than in NCR. The resistance curves shown in Fig. 3 reveal that this was not only at maximal NA values, but also throughout the whole



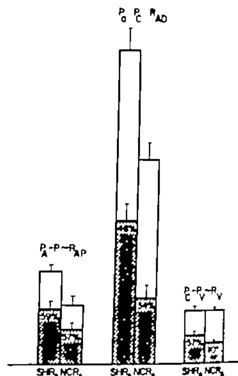


Fig 1 Fig. 2

Fig. 1 Section A compares the effect of normal and low Ca_e on the R_T responses to NA in NCR, RH SHR$_A$ and SHR$_Y$. To facilitate the comparison the maximum R_T response at normal Ca_e in NCI expressed as 100% All other values re expressed as percentage of NCR control values, with each hy tensive group being compared to ts own matched control group.

Section B compares the same maximal R_T responses to NA at normal and low Ca_e^2 in NCR, RHR, SH and SHR$_Y$ but now with each R_T at normal Ca_e expressed as 100%. This allows for a more direct co parison of the extent of reduction t low Ca f the maximum R_T response t Na in NCR RHR, SH and SHR$_Y$. Note that both RHR d SHR$_A$ differ significantly from NCR but in opposite directions

Fig. 2. Shows the maximum resistance responses t NA i consecutive sections of the hindquarter exc bed at normal and low Ca Results re expressed as mmHg pressure with hatched areas represent responses at low Ca_e expressed as percentage of those t normal Ca_e R_{AP} denotes resistance to fl in proximal precapillary (rterial) resistance vessels, R_{AD} that in distal precapillary resistance ve and R_V that in the postcapillary (venous) resistance vessels

resistance than in the local myogenic tone, the latter being predominantly a function the distal precapillary resistance vessels

During constant flow perfusion and NA infusion, R_{AP} also increased approximat to the same extent as during enhanced vasoconstrictor fibre discharge. Thus, at maxir NA vasoconstriction the proximal precapillary resistance vessels contributed 19 and 21 respectively of the total resistance in NCR$_A$ and SHR$_A$ (*cf* Fig. 5) At *low* Ca_e the N responses of the proximal precapillary resistance vessels were essentially *equally* reduc in NCR$_A$ and SHR$_A$ When expressed in terms of the maximal $P_A - P$ difference, achiev during supramaximal NA infusion during low Ca_e 57 and 59% of the $P_A - P$ respons at normal Ca_e remained as illustrated in Fig. 2. If one instead considers the R_{AP} respon in terms of the $P_A - P$ difference after subtracting the $P_A - P$ difference at complete vascul relaxation (thus expressing the extent of smooth muscle shortening) almost exactly 50

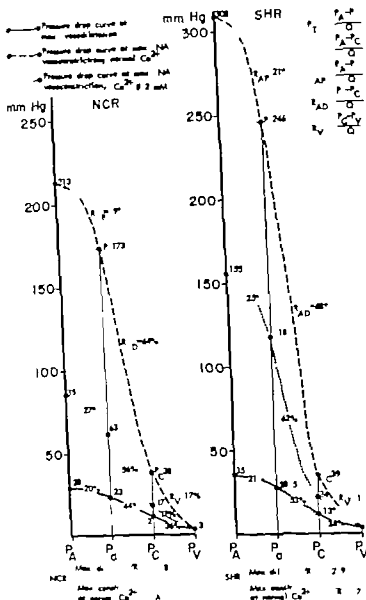


Fig. 5 A diagrammatic representation is show of the pressure drop along the consecutive vascular segments of the perfused hindquarters at NCR and SHR_A during 1) complete vascular relaxation, 2) maximal NA concentrations at low calcium and 3) maximal NA concentrations at normal calcium.

of resistance vessels (R_T in comparison with R_{AP}, R_{AD} and R) to induce the same degree of vasoconstriction as that produced by 7.5–10⁻ mol/l of NA (0.15 g/ml) on the NCR_A vessels at normal Ca. The Figure expresses in another way what has been stated above, i.e. that while there was no significant difference between NCR and SHR_A as to the main-

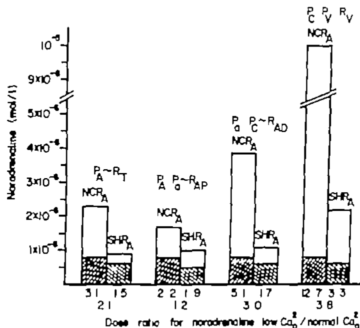


Fig. 4. Concentrations of … adrenaline which are equipot… to the concentration of 7.5+1… mol/l (0.15 μg) in NCR at nor… Ca_o^{2+} Consecutive vascular … tions NCR_A and SHR_A at low (… mM open columns) and nor… (>1.5 mM hatched colu… Ca_o^2 are compared.

range of NA concentrations. This fact will of course, greatly increase confidence concern… differences observed than if only the effect of one NA concentration had been test… This also holds true for all the other comparisons discussed above. This Figure also allow… a comparison of the NA concentration needed to produce identical pressor (resistance… responses in SHR_A and NCR_A both at normal and low Ca_o (*cf* Fig. 4). From the mode… NCR_A response to 0 15 μg/ml of NA at normal Ca (dotted line), it can be seen that th… NA concentration must be increased relatively much more in NCR_A (from 0.15 to 0.8… *i.e.* some 5-fold) than in SHR_A (from 0 12 to 0.20· *i.e.* about 2-fold) to produce the sam… pressor response at low Ca_o^+ (see arrows) Thus, when compared in this way it is also cle… that the distal pre-capillary vessels maintained their contractility to NA much better i… SHR_A than in NCR_A at low Ca_o

R_V The right part of Fig. 2 shows the maximal venous resistance responses to NA … normal and low Ca_o in SHR_A and NCR_A In contrast to the precapillary resistance vesse… the venous resistance vessels did not differ significantly at normal Ca_o but, again, the SHR… response was better maintained than the NCR_A one at low Ca_o This was the case wheth… one, as here, depicts the highest $P_O - P_V$ difference achieved at maximal NA concentratio… or if one first subtracts from this value the $P_O - P_V$ difference present at complete vascul… relaxation. The former reflects the maximal contractile strength of the venous resistanc… vessels, the latter rather reflects their degree of shortening, but confronted with a rising… transmural pressure thanks to the constant flow Both ways of describing the venous re… sponses have their justification and both reveal a less well maintained response in NCR_A… compared with SHR_A at low Ca_o

III *Integrated R_T, R_{AP}, R_{AD} and R_V responses in SHR_A and NCR_A*

In Fig. 4 a comparison is made of the difference between SHR_A and NCR_A with respect to how much the Na concentration has to be increased for the various consecutive sections

ormally responsible for myogenic tone and are also an important part of neurogenic ol, seem to be almost entirely dependent on Ca_o for smooth muscle activation in the otensive rat (Sutter *et al* 1977). Therefore, these important microvessels show a closer onship to the smooth muscle of the portal vein than to that of the aorta. The present s show that resistance vessels of SHR and RHR in this respect differ but in opposite tions, from the resistance vessels of the normotensive controls with regard to their ndence on Ca_o for contractility to the adrenergic transmitter NA. Thus, the SHR ls exhibited better maintained NA responses at low Ca_o than the NCR vessels but the rence was only clearly obvious in the well-established phase of hypertension in SHR_A, there was only a tendency in this direction in SHR. These results are in general ement with recent findings *in vitro* on subcellular fractions of SHR mesenteric arteries, h indicate a better calcium storing capacity in SHR vascular smooth muscle (Wei, and Daniel 1976). The most reasonable explanation of the present findings concerning true resistance vessels in SHR is that they have an increased amount of calcium available e contractile machinery. In RHR, on the other hand, the NA responses of resistance els were more depressed in low Ca_o than those of the NCR, as though the smooth cle cells per unit mass retain or possess smaller calcium stores in RHR than in NCR. of interest that the resistance vessels of the genetically hypertensive rats of the New land strain (GHR) in this respect seem to be more related to the RHR vessels than to SHR ones, to judge from findings on the GHR mesenteric vessels (Jones, Lyon and ian 1973).

t normal levels of external Ca both SHR and RHR show the same type of vascular erreactivity and it is consistent with altered wall to lumen ratios. For these reasons the cular hyper-reactivity of both RHR and SHR, as revealed in the perfusion study, seems be entirely matter of structural precapillary adaptation of the vascular bed (*cf* Folkow al. 1973). Furthermore, the fact that the difference between SHR and NCR concerning dependence paralleled, rather than preceded, genetically-linked primary hypertension rdly suggests that this changed Ca_o dependence reflects any primary trigger element terms of an altered excitation-contraction coupling preceding the hypertensive state. this respect, there is clear contrast, *cf* to the enhanced central nervous "hyperreactivity" SHR, which is particularly marked in the early "prehypertensive" stage, and *via* neuro-rmonal links then brings about an enhanced excitatory drive on the SHR cardiovascular stem (*Hallbäck and Folkow 1974*).

Nevertheless, it is clear that an alteration of the cellular handling of Ca^{++} has, indeed, ken place in SHR_A, consistent with an increase in available calcium, either intracellular membrane-bound, and that the situation in this respect is quite different in RHR and that oth differ quantitatively from NCR. The simplest explanation of the present findings in HR is that the vascular smooth muscle effectors, along with their functional, chemical nd structural adaptation in response to the enhanced neurohormonal excitatory drive, ho develop an improved capacity for the extracellular stores to hold calcium, consistent ith the findings of Wei, Janis and Daniel (1976). Tentatively this could be due to develop-ent of the sarcoplasmic reticulum to an extent that more than matches the increased ormation of contractile filaments that occurs in connection with smooth muscle hyper-
r

tenance of the NA response in the R_{AP} section at low Ca_o^+ the responses in the R_A R_V sections were much better maintained in SHR_A than in NCR_A at low Ca_o and th consequently also the case for R_T (*cf* Folkow *et al* 1976). The difference appears particularly marked for the venous resistance vessels, which was due to the fact th NCR_A dose–response curve was markedly displaced to the right at low Ca_o^{++}

In Fig. 5 the present results on SHR_A and NCR_A are schematically summarize different way *i.e* as the profile of pressure drop along the hindquarter vascular bed, at complete vascular relaxation and at maximal NA vasoconstriction at either n (> 1 5 mM) or low (0.2 mM) Ca_o^+ This way of illustration perhaps gives the best impr of the marked differences between NCR_A and SHR_A in terms of their peripheral hemody ics, which ensue from the different design of their precapillary vessels, where those of S are characterized by narrowed lumina and a relatively thicker media, *i.e* an incr w/r_i Besides having a considerable influence already during maximal dilatation, this a vascular architecture further implies a marked precapillary "hyperreactivity" duri creasing vasoconstriction in SHR_A and the end-result of this is shown in the Figure a maximal NA vasoconstriction at normal Ca_o Fig. 5 also illustrates the relatively g dependence on Ca_o of the NCR_A resistance vessels compared with the SHR_A ones by of the pressure drop curves at low Ca_o but where the vessels were again exposed to ma NA concentrations. These curves are based on the "weighed" mean values from a $SHR_A - NCR_A$ comparisons performed in the present study and in general these r closely resemble those of an earlier analysis of the pre- and postcapillary resistance resp in SHR and NCR hindquarters performed on animals of about the same age (Folkow 1974)

Discussion

The present experiments were aimed at exploring some aspects of excitation–contra coupling in the systemic resistance vessels by comparing the dependence on Ca_o^+ lo responsiveness to noradrenaline (NA) if the hindquarter vascular beds of normoto (NCR), renal hypertensive (RHR) and spontaneously hypertensive rats (SHR). The que was whether these types of secondary and genetically linked, primary hypertension, re tively differ mutually and in relation to normotensive controls, as to vascular contrac to NA when the Ca_o concentration is drastically lowered

It is well-known, as outlined in Introduction, that various types of muscle can markedly in terms of their intrinsic Ca_i stores and dependence of Ca_o and this is the even when different parts of the systemic vascular bed are compared, such as aortic portal vein smooth muscle (Sutter 1976) Furthermore it is much debated whether an al tion in the intracellular handling of calcium, and hence of the excitation-contraction pro might be one of the basic phenomena behind the changed control of the enhanced resist If for example, such an altered calcium handling were one of the initiating mechan behind the enhanced resistance, and, furthermore, was common to all types of hyperten it might already be expected to reveal itself in the early phases of hypertension an exhibit similar signs of alteration independent of how the hypertensive state was initiat

It has recently been shown that the resistance vessels closest to the capillary level, w

creased stores of intracellular Ca^{++}. Neither is there any *a priori* reason to imagine al mechanisms on the cellular-subcellular level behind these two different types ertension.

s, by no means, denies that the excitation–contraction coupling remains an indispens- nk in the whole process of cardiac and vascular contraction, but from this it does not that "primary" changes in this coupling process should necessarily constitute a d starting process, either in SHR or RHR (or GHR) hypertension. At least the present lend no support to such a view though they do *indicate that changes in the contraction* ing must have taken place both in SHR and RHR. These changes may well be of a dary nature and reflect different ways of effector cell adaptation to the new situation.

udies were supported by grants from the Swedish Medical Research Council (No 14X-00016), Columbia Heart Foundation and the British Medical Research Council (J. V. Jones was British Travelling Research Fellow for 1975–76). AB Hässle generously covered part of the expenses for ation. — The expert technical assistance of Gunnel Andersson, Ulla Axelsson and Gertrud Karlsson tefully acknowledged.

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trophy Such enhanced cellular stores of calcium are, however evidently also pres
the postcapillary smooth muscles, since SHR also differs here from NCR, despite th
that the venous side hardly seems to be exposed to any increased pressure and does not
any clear signs of either true media hypertrophy or any hyper-reactivity concerning the v
resistance responses. For such reasons also, the suggested enhancement of the intrac
calcium storage is *not* a likely explanation for the resistance vessel hyperreactivity in
as this seems to be confined to the precapillary resistance vessels only (*cf* Folkow
1974) at least in the hindquarters. It should be noted, however that Simon (1976
reported altered capacitance function in SHR, but this may partly represent other v
sections than the postcapillary resistance ones, and their characteristics are not neces
identical Further the present findings show that also the proximal precapillary resis
vessels displayed hyperreactivity but they did not differ from those of the NCR as to
Ca_e

Thus, it seems logical to assume that the characteristic pre-capillary hyperreac
in SHR is mainly due to the structurally increased wall/lumen ratio of these vessel
more so as the same type of hyper-reactivity is seen in the similarly altered RHR ve
In the RHR vessels the intracellular stores of calcium appear as mentioned, to be rela
reduced compared with normotensive controls, possibly because the increased form
of contractile filaments within the muscle cells is here not matched with a correspo
increase of sarcoplasmic stores of calcium. Why the RHR (and evidently also GH
mentioned above) smooth muscle cells differ from the SHR ones in this latter resp
not known. *A priori* it might reflect either a genetically-linked difference in smooth m
behaviour and adaptation to the increased activation and load. It is also possible tha
different types of excitatory influences in SHR and RHR (and GHR) imply differences
concerning so-called "trophic influences, that seem to modify the structural adapt
of the cardiovascular effector cells when exposed to the high pressure state (*cf* Fo
1975)

Whatever the case, it seems, at least from the point of view of the present findings,
the same mechanisms of altered intracellular handling of calcium are not crucial fo
initiation and maintenance of both the SHR and the RHR hypertension. If altered han
of calcium is involved in RHR, it is manifest only at normal levels of external calc
Both types of hypertension may so far be adequately explained by the combined influ
of the above-mentioned extrinsic excitatory influences on the cardiovascular effector
and their rapidly developed "structural autoregulation" which tends to form a pos
feedback interaction with such influences (*cf* Folkow 1975) Possibly the relatively l
intracellular storage capacity of the RHR vessels smooth muscle might imply difficu
for these cells to rapidly enough eliminate the increased influx of Ca_e upon excitat
which might help to prolong and enhance their contractile response, other things b
equal. After all, our earlier study in NCR (Sutter *et al* 1977) suggests that most of the C
needed for the contraction of the true resistance vessels has to be derived from the exte
environment of the cells, and then they are faced with the time problem of getting rid of
intracellular Ca concentration rapidly enough to allow for relaxation. The same tenta
explanation cannot possibly be used also in SHR, which behave as if their blood ves

Methods

...erative procedures. The experiments were performed on 12 cats, weighing 2.2–3.8 kg, fasted for ... 4 h. ...animals ...ere anaesthetized ... ith chloralose (50 mg/kg) after induction ...ith ether. A tracheal cannula ...serted to allow ... free airway. The abdomen ...as opened in the midline and the greater omentum, the ...intestine and the spleen were extirpated.
...animals ...ere heparinized (3 mg/kg). A femoral artery ...as cannulated and connected to a mercury ...meter for continuous recording of arterial blood pressure.
Blood flow recordings. T... record the magnitude of the overall colonic blood flow ... ide-bore poly-...ene tube ...as inserted into the superior mesenteric vein and the venous outflow was diverted to ...d perspex drop chamber filled ...ith silicon oil, and returned to the animal via the external jugular veins. ...drop counter operated an ordinate ...rater recording on smoked kymograph paper, the height of the ...mes being inversely proportional to the rate of the blood flow (see figures). For technical reasons the ...us blood flow in some animals had to be determined by arterial inflow measurement. For this purpose ...de polyethylene tube ...as inserted in the left carotid artery and the blood flow ...as diverted into the ...recorder unit, in turn connected to the proximal end of the centrally cut superior mesenteric artery ...inferior mesenteric artery ...as in these experiments severed, ... procedure that has been demonstrated ...o alter total colonic blood flow in the cat (Hultén 1969). Irrespective of the method used the regional ...d flow ...as qualitatively and quantitatively the same, and by using arterial inflow recording close intra-...rial infusion of drugs (see below) ...as greatly facilitated. I... all experiments the periarterial vasocon-...tor fibres along the superior and inferior mesenteric artery were dissected free and severed.

Mechanical stimulation. Mechanical mucosal stimulation ...as performed by pulling ... slightly wetted ...-swab by rotating movements through the colonic lumen. The gauze-swab was kept small enough not ...use distension of the colonic ...all. The "stimulator" ...as inserted into the lumen either through ... small ...ion in the proximal end or at the midpoint of the colon. The latter method ...as chosen when the effects ...mechanical stimulation of the proximal and distal half of the colon was to be studied separately. I... some ...ements ... small latex balloon ...as also used for studying the effects of distension. For this purpose the ...oon was rapidly inflated ...ith air to ... pressure of about 20 mm Hg.

...) Nervous stimulation. I... all but 4 expts. the pelvic nerves ...ere dissected free on both sides and divided ...re they emerged from the sacral roots. The distal ends ...ere mounted on silver-ring electrodes for ...sequent stimulation. In 6 expts. the distal cut ends of the periarterial vasoconstrictor fibres along the ...erior and inferior mesenteric arteries were also mounted on electrodes for studying the effects of mech-...cal stimulation during sympathetic nervous stimulation. Supramaximal square ...ave pulses ...ere de-...ered from ... Grass Stimulator (model S 5 E) to the pelvic nerves (8 V, 5 ms) and the sympathetic bundles ...V, 5 ms). Generally 4–1... Hz were used, ...hich corresponds t... the upper range of the physiological ...charge rates.

E. Administration of drugs. Atropine sulphate ...as administered ... in ... dose of 1 mg/kg. Close i.a. ...ministration of 5-HT (5-hydroxytryptamine, SIGMA, Chemical Co.) and dihydroergotamine (DHE, ...Sandoz) into the superior mesenteric artery were either (venous outflow recordings) performed ... cannula inserted into ... mesenteric arcade artery or (arterial inflow recordings) via the polyethylene ...be between the drop recorder and the superior mesenteric artery. 5-HT ...as administered either as bolus ...ections or as continuous infusions ...ith an infusion pump. DHE, blocking the effects of 5-HT, was ...ven as ... bolus injection in the superior mesenteric artery ...hile occluding the supplying artery. When ...leasing the occlusion venous blood ...as collected for 1–2 min to reduce the amount of DHE reaching the ...neral circulation. In order to obtain blockage of the adrenergic alfa- and betareceptors, phenoxybenzamine ...(Dibenyline, Smith, Kline & French Lab. Ltd.) 5 mg/kg and propranolol (Inderal® ICI-Pharma) 3 mg/kg ...ere given. ... The blocking effects ...ere tested by means of sympathetic nerve stimulation (alfa-blockade) ...and isoprenaline (beta-blockade).

Results

1. The effect on colonic blood flow of mechanical stimulation of the mucosa

With the pelvic nerves bilaterally severed mechanical mucosal stimulation of the proximal colon either by means of friction stimuli or distension elicited a marked blood flow increase amounting to about 50 per cent of control flow (Fig. 1 left panel). The blood flow increase,

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Vascular Responses to Mechanical Stimulation of the Mucosa of the Cat Colon

By

S FASTH L. HULTÉN O LUNDGREN and S NORDGREN

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Abstract

FASTH, S L. HULTÉN O LUNDGREN and S NORDGREN *Vascular responses to mecha stimulation of the mucosa of the cat colon* Acta physiol scand 1977 *101* 98 104.

Mechanical stimulation of the mucosa of the proximal colon evoked hyperemia hich also cou elicited when severing the ut nomic fibres to the colon. Mechanical stimulation of the distal colon duced hyperemia which, on the other hand, was abolished by cutting the pel ic nerves. Afferent p nerve stimulati n evoked a response similar to that seen when stim lating the distal colon Adre and cholinergic recept blockers did not affect the studied colonic asodilatations. Dihydroergota given i doses abolishi g the ascular effects of i.a. injected 5-hydroxytryptamine bolished the hyper to mechanical stimulation of the proximal colon but not that of the pelvic stimulation. It is conclude a local nervous vasodilator reflex exists the proximal colon simila to that earlier demonstrated i small bowel (B ber *et al* 1971) and that the reflex vasodilatation evoked by mechanical stimulation o distal colon is mediated via the pelvic nerves. Hence, the vascular control differs in the proximal and d distal parts of the colon

Recent studies on vasodilator mechanisms in the small intestine of the cat have provi evidence that mechanical stimulation of the jejunal mucosa induces a substantial trans intestinal vasodilatation which is probably transmitted via an intrinsic nervous reflex i dependent on the release of 5-hydroxytryptamine (5-HT) (Biber Lundgren and Svan 1971 Biber Fara and Lundgren 1974). Furthermore, this vascular response is neither bloc by cholinergic or adrenergic blocking agents, nor by ganglionic blocking agents (Biber e 1971). It has also previously been shown that mechanical stimulation of the rectal muc gives rise to a pronounced transient vasodilatation in the colon (Hultén 1969), but t sacrospinal reflex response, which is associated with a colonic contraction is dependent intact pelvic nerves. Moreover both the motor and the vasodilator responses are atrop resistant and the transmittor mechanisms involved are unknown.

The aim of the present study was to explore if a local vasodilator response similar to t in the small intestine could be elicited by mechanical stimulation also of the colonic muc and to study the role of 5-HT in the reflex vasodilatation associated with mechanical stimu tion of the rectal mucosa.

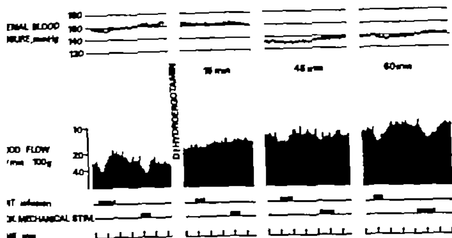


Fig. 2. Cat 3.8 kg. The vasodilatation induced by mechanical mucosal stimulation and intraarterially infusion of 5-HT before and at varying intervals after giving DHE. Note that the vascular responses which are completely blocked after 15 min, gradually return to reach control level 60 min after DHE administration.

The effects of a pharmacological 5-HT blockade on the studied vasodilator responses

The vasodilatations induced by mechanical stimulation and pelvic nerve stimulation were studied before and after giving dihydroergotamine (DHE). To test the effect of this blockade, 5-HT was injected or infused into the colonic circulation before and after DHE administration. Administration of 5-HT either as a bolus injection (10 µg) or infusion (50–100 µg/min), evoked a vasodilatation of the same type as that produced by mechanical mucosal stimulation. As is seen in Fig. 2, DHE (1 µg/kg) administered as a close i.a. injection during mesenteric circulatory arrest, completely abolished this vasomotor effect.

During such a 5-HT blockade mechanical stimulation failed to produce any vasodilatation (Fig. 2). However the shortlasting vasoconstriction previously described as an initial effect of mechanical stimulation remained and was in fact sometimes prolonged, outlasting the period of stimulation (see panel C of Fig. 3).

The 5-HT blockade remained efficient for some 40 min but after that period the vasodilatation elicited by stimulation of the proximal colonic mucosa exhibited the same magnitude as before the blockade. In this situation the vasomotor effects of 5-HT could also be reproduced (Fig. 2). The vasodilatation evoked by pelvic nerve stimulation was not abolished, however (Fig. 3) and there was still a sustained colonic contraction, indicating that both the vasodilator and motor responses on pelvic nerve stimulation were unaffected by the 5-HT blockade.

The effects of mechanical stimulation during activation of sympathetic nerves and during alfa- and beta-adrenergic blockade

When mechanical stimulation of the proximal colonic mucosa was performed during continuous vasoconstrictor fiber stimulation the same vascular response as during control conditions was observed though of a lesser magnitude. The peak reduction of the vascular

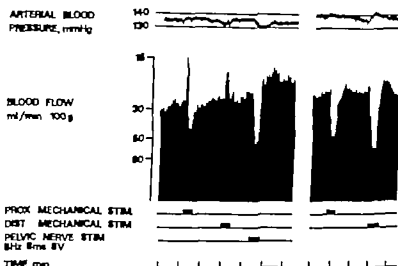


Fig. 1 *Left panel.* Cat 2.3 kg (severed pelvic nerves). The effect of proximal and distal mucosal stim as compared to that of efferent pelvic nerve stimulation on colonic blood flow Note that the bloo increase could only be elicited on mechanical stimulation of the proximal colon.

Right panel. Cat 3 1 kg (intact pelvic nerves) The effect of proximal and distal mucosal stimulatio colonic blood flow Note that distal stimulation now evokes a blood flow increase, indicating a refle through the pelvic nerves.

often preceded by a transient decrease, reached its maximum within 30 s after comm ment of the stimulation and returned to control level 1–3 min following its cessation. blood flow response could be reproduced throughout the experiment. When the muco the resting proximal colon was just pinched without any stretching of the mesentery o colonic wall, the same flow response was evoked. In constrast neither mechanical mu stimulation nor distension of the distal colon caused any increases in the blood flow though sometimes a shortlasting decrease of blood flow was seen (Fig. 1 left panel).

Efferent pelvic nerve stimulation induced the earlier reported pronounced colonic dilatation (Fig. 1 left panel) When comparing this blood flow response with the one eli by mucosal stimulation a difference was observed. Although there was a pronounced b flow increase occurring within some 5–10 s after commencement of the nervous stimula reaching its maximum within 20–30 s, this blood flow increase vanished within 30– and there was also a sustained motor contraction of the colon. This was never observed proximal mechanical mucosal stimulation or distension, although an augmented ryt peristalsis was occasionally observed

In experiments with the pelvic nerves intact, mechanical stimulation of the prox colonic mucosa gave rise to a blood flow increase which was similar to that observed w the pelvic nerves were severed Mechanical stimulation of the distal colonic mucosa produced a blood flow increase which was in some experiments more marked than caused by proximal stimulation (Fig. 1 right panel) The shortlasting blood flow decr which was often observed on proximal mucosal stimulation was also observed on d mucosal stimulation. Simultaneously with the vanishing blood flow increase, occur within 30–40 s, there was also a sustained motor contraction, similar to that seen on pe nerve stimulation.

mucosa of the proximal colon is mediated by a local nervous reflex as in the small bowel (ber *et al.* 1971, 1974). The vasodilatation observed upon stimulating the distal colon was -arently the same as the hyperemic response earlier reported by Hultén (1969).

A shortlasting vasoconstriction was often observed just before the hyperemic response on mucosal stimulation. A similar response pattern was reported by Biber *et al.* (1971) en mechanically stimulating the small bowel mucosa. These authors ascribed this effect a stimulation artefact caused by the handling of the intestinal segment before stimulating mucosa. In the present study, however, the initial vasoconstriction appeared even when iching the mucosa which could not possibly exert a mechanical interference with the ous outflow. Neither alfa- and beta-blocking agents, nor DHE had any influence on this soconstriction which seems to be caused by some unknown mechanism.

Dihydroergotamin (DHE), in doses blocking the effect of i.a. injected 5-HT, was the only armacological agent tested in this study which could abolish the vasodilatation evoked stimulating the mucosa of the proximal colon. The intestinal wall contains large amounts 5-HT, which is mainly located in the enterochromaffine cells (Vialli 1966, Penttilä and rmpinen 1968) and also, according to some authors, in some of the nerve cells that contures the intramural plexa (Gershon and Ross 1968). The physiological significance of ese 5-HT stores is largely unknown (Furness and Costa 1973). In view of the present dings and earlier reports on the effects of mechanical stimulation on jejunal blood flow the cat (Biber *et al.* 1971, 1974, Biber 1973), it seems possible that 5-HT, released by even ght stimulation of intestinal mucosa, plays an important role in the functional hyperemia the gut at least in the small bowel and the proximal colon. From a theoretical point of ew it appears attractive t assume that local factors are involved in the control of a func onal mucosal hyperemia producing localized blood flow increases to meet with increased utritional demands in different parts of the intestine during the digestive work.

While the results of the present study seem to indicate that 5-HT is probably involved in e functional hyperemia of the proximal colonic mucosa, this is apparently not the case in e distal colonic part, where another vasodilator mechanism is operating. As has been reviously demonstrated (Hultén 1969) this part of colon is controlled by the pelvic parasympathetic nerves. Activation of these nerves gives rise to a marked mucosal hyperemia, associated with an increased secretion and a tonic motor contraction. The motor and vascular responses are atropine-resistant and direct and indirect evidence favours the presence of a kinin-l ke mechanism (Fasth and Hultén 1973, Fasth 1973, Fasth, Hultén, Johnson and Zeitlin 1976).

Earlier studies have shown that nervous effects exerted on the musculature and on the vascular bed are differently organized in the proximal and distal half of the colon and that this differentiation might ha e important functional implications (Hultén 1969). Thus the proximal part of the colon exhibits a characteristic motility pattern which favours absorption, whereas the motility pattern seen in the distal part would rather favour storage and expulsion. Moreover, the proximal part differs from the distal part insofar that it is devoid of centrally controlled vasodilator fibres. In many respects therefore the proximal colon is functionally ery similar to the small intestine, and the results of the present study lend further support to the view that the colon is comprised of two functionally different units.

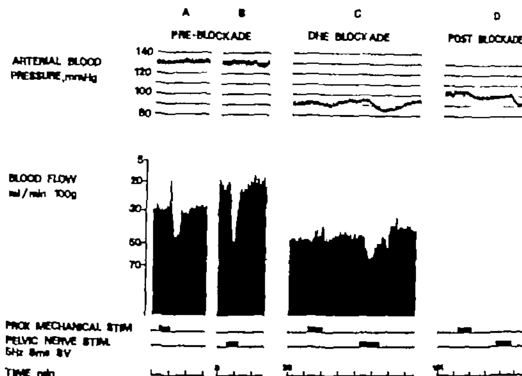


Fig. 3 Cat 2.3 kg. The effect of DHE-blockade on the colonic blood flow responses to proximal mech stimulation and to efferent pelvic nerve stimulation. Panels A and B show the responses before the bloc Panel C shows the effects during DHE-blockade. Note that the blood flow increase evoked by me stimulation of the proximal colon is completely abolished during the DHE-blockade, wheres the of pelvic nerve stimulation is virtually unchanged. Panel D shows the returning response of pro mucosal stimulation about 1 h after DHE was given

resistance amounted to about 20 per cent compared to a maximal reduction of aro 50 per cent during prestimulatory control. Neither propranolol (3 mg/kg i.v.) nor phe xybenzamine (5 mg/kg i.v.) affected the vascular response to mechanical stimulation.

Discussion

The present study has demonstrated that stimulation of the mucosa of the proximal par the acutely denervated colon produced a marked hyperemia while no vasodilatation seen when stimulating the distal part of the denervated colon. In the innervated large bo vasodilatations were evoked by mechanical stimulation in the proximal as well as in distal parts of the colon. The hyperemia seen in the proximal colon exhibited all the char teristic features earlier reported by Biber *et al* (1971 1974) with regard to the vasodilatat evoked by mechanical stimulation of the small intestinal mucosa. Hence, the vasodilatati preceded by a shortlasting vasoconstriction, could be induced in the denervated colo Furthermore, the hyperemia was not blocked by adrenergic or cholinergic receptor blocki agents. However it could be abolished by administration of a 5-HT receptor blocking age Although the effect of a nerve conductivity blocking substance such as *e g* tetrodotox was not tested in this study it is proposed that the vasodilatation observed when stimulati

the mucosa of the proximal colon is mediated by a local nervous reflex as in the small bowel (Biber *et al.* 1971 1974). The vasodilatation observed upon stimulating the distal colon was apparently the same as the hyperemic response earlier reported by Hultén (1969).

A shortlasting vasoconstriction was often observed just before the hyperemic response upon mucosal stimulation. A similar response pattern was reported by Biber *et al.* (1971) when mechanically stimulating the small bowel mucosa. These authors ascribed this effect to a stimulation artefact caused by the handling of the intestinal segment before stimulating the mucosa. In the present study however the initial vasoconstriction appeared even when stroking the mucosa which could not possibly exert a mechanical interference with the venous outflow. Neither alfa- and beta-blocking agents, nor DHE had any influence on this vasoconstriction which seems to be caused by some unknown mechanism.

Dihydroergotamin (DHE), in doses blocking the effect of i.a. injected 5-HT was the only pharmacological agent tested in this study which could abolish the vasodilatation evoked by stimulating the mucosa of the proximal colon. The intestinal wall contains large amounts of 5-HT which is mainly located in the enterochromaffine cells (Vialli 1966, Penttilä and Lempinen 1968) and also, according to some authors, in some of the nerve cells that constitutes the intramural plexa (Gershon and Ross 1966). The physiological significance of these 5-HT stores is largely unknown (Furness and Costa 1973). In view of the present findings and earlier reports on the effects of mechanical stimulation on jejunal blood flow in the cat (Biber et al. 1971 1974, Biber 1973), it seems possible that 5-HT released by even slight stimulation of intestinal mucosa, plays an important role in the functional hyperemia of the gut at least in the small bowel and the proximal colon. From a theoretical point of view it appears attractive to assume that local factors are involved in the control of a functional mucosal hyperemia producing localized blood flow increases to meet with increased nutritional demands in different parts of the intestine during the digestive work.

While the results of the present study seem to indicate that 5-HT is probably involved in the functional hyperemia of the proximal colonic mucosa, this is apparently not the case in the distal colonic part, where another vasodilator mechanism is operating. As has been previously demonstrated (Hultén 1969) this part of colon is controlled by the pelvic parasympathetic nerves. Activation of these nerves gives rise to a marked mucosal hyperemia, associated with an increased secretion and a tonic motor contraction. The motor and vascular responses are atropine-resistant and direct and indirect evidence favours the presence of a kinin-like mechanism (Fasth and Hultén 1973, Fasth 1973 Fasth, Hultén, Johnson and Zeitlin 1976).

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Acta physiol. scand. 1977. 101. 105–111

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Reflex Plasma Hyperglycemia and Hyperosmolality Evoked by Unloading of the Carotid Baroreceptors

By

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Abstract

JÄRHULT, J., J. HOLMBERG and J. LUNDVALL. *Reflex plasma hyperglycemia and hyperosmolality evoked by unloading of the carotid baroreceptors.* Acta physiol. scand. 1977. *101.* 105–111.

Hemorrhage is usually accompanied by a considerable increase in the plasma osmolality and glucose concentration due to an augmented release of glucose from the liver. In the present cat experiments an attempt was made to investigate the possible role of different vascular receptors in mediating this hyperglycemic (hyperosmolar) response. Bilateral vagotomy or stimulation of the carotid chemoreceptors by perfusion of the carotid sinus with venous blood at normal pressure only slightly increased the arterial plasma glucose concentration. On the other hand, when the sinus nerves were cut in the vagotomized animal, thereby simulating complete unloading of the carotid baroreceptors, the arterial plasma glucose concentration rose by about 6 mM/L and the arterial plasma osmolality by about 10 mOsm/kg H_2O. Perfusion of the carotid baroreceptors with arterial blood at different levels of hypotension showed that the baroreceptor-induced hyperglycemia was graded in relation to the pressure level. Regional hypotension of the liver, pancreas, intestine, kidneys or brain did not significantly affect plasma glucose concentration or osmolality. We conclude that the reflex release of glucose from the liver during hemorrhage mainly is initiated from the unloading of arterial baroreceptors.

It has been known for some years that hemorrhage is associated with a pronounced increase in the plasma osmolality (*e.g.* Bergentz and Brief 1965, Baue *et al.* 1967, Boyd and Mamberger 1968). Recent studies from this laboratory have demonstrated that the hyperosmolality during acute hemorrhagic hypovolemia can be mainly attributed to hyperglycemia (*cf.* Bernard 1877, Robertson 1935, Brooks *et al.* 1963) and, further, that it contributes in an important way to the plasma volume restoration by causing a glucose-osmotic transcapillary absorption of fluid from the extravascular compartment into the blood stream (*e.g.* Järhult *et al.* 1972, Järhult 1973, 1975 b, Järhult *et al.* 1976). The hyperglycemia also seems to expand the overall extracellular fluid volume in bleeding by producing glucose-osmotic fluid withdrawal from the intracellular to the interstitial space (Järhult 1975 b). The hyperglycemia is established by an increased release of glucose from the liver (*cf.* Schenk 1894) and seems to be mediated by several different links of the sympatho-adrenal system, *i.e.* by a direct adrenergic influence on the liver cells and,

indirectly by adrenergic control of catecholamine and glucagon release from the adr and pancreas (Järhult 1975 a)

The direct adrenergic effects on the heart and the blood vessels in hemorrhage are k to be initiated from several receptor areas in the circulatory system (*e.g.* Heymans Neil 1958 Öberg 1964 Chien 1967), but the receptors mediating the metabolic hy glycemic response to bleeding have so far not been identified. An attempt was ma the present investigation to analyse the possible role of certain cardiovascular recepto this hyperglycemic response to hemorrhage.

Methods

30 cats (mean b.wt 3.2 kg) were used in the study The animals fasted 12 14 h before the experime received water *ad libitum*. They were anesthetized i.v with chloralose (50 mg/kg) and urethane (5 mg/kg) after induction with ether A tracheal cannula was inserted a d the animal breathed spontane throughout the experiment. Heparin (1 000 IU/kg b.wt.) was given prior to intravascular cannulation brachial artery was cannulated for recording systemic arterial pressure and for sampling of arterial b Heart rate was recorded with a Grass tachograph triggered by the systolic pressure wave. Muscle flow was estimated in some of the experiments by measuring with a optical drop recorder the outfl blood flow from the cannulated right femoral vein. In these expts. the right paw was removed external pressure applied to the thigh so that flow through venous vessels other than the cann femoral vein was prevented without interfering with the arterial inflow

In an attempt to reveal the possible role of various receptors in the circulatory system in the h glycemic (hyperosmolar) response to hemorrhage, arterial plasma glucose concentration and osmo were followed in the normotensive animal during alterations of receptor activity resembling qualit those occurri g in bleeding Fo this purpose, the carotid chemoreceptors were stimulated and the ca baroreceptors unloaded in cat after bilateral cervical vagotomy The effects of vagotomy *per se* ere studied. The possible influence on plasma glucose concentration and osmolality from postulated med receptor sites in the renal, splanchnic d cerebral circulations was investigated as well (see Discuss

The carotid chemoreceptors were stimulated according to the technique described by Little and C (1975). The right iliac artery and vei were cannulated with polyethylene tubings nd the arterial venous flows diverted through two separate channels of Harvard peristaltic pump Distal to pump arterial and venous tubi gs joined to a common tubing which after bifurcation was connected to the commo carotid arteries. All main arterial vessels in the vicinity of the carotid body were then lig except f the external carotid arteries which were can ulated and the outflows diverted to a funnel right external jugula vein. The carotid intra sinusal pressure was recorded bilaterally via T tubes i external carotid arteries and could be controlled by means of screw clamps placed distal t the T-t With this technique for artificial flow perfusion, the chemoreceptors in the isolated carotid sinus-gl regions could be stimulated by switchi g from arterial t venous blood perfusion under circumstanc constant, normal pressure in the carotid sinus. The normotension was accomplished by adjustments o peristaltic pump nd the screw clamps.

The carotid baroreceptors were inactivated in vagotomized cat with two different techniques. (1) A c plete unloading of the baroreceptors was simulated by cutting the sinus nerves bilaterally in the other intact sinus regions. (2) The above described preparation for rtificial perfusion of the carotid inus re was used for graded inactivation of the baroreceptors. For this purpose, the sinus regions were perf with arterial blood at mean inflow pressures of bout 120, 90, and 60 mmHg, respectively

Local splanchnic hypotension was produced with adjustable screw clamp placed on the coelia superio mesenteric artery Regional blood pressure was recorded in the left gastric artery or in a s jejunal artery Local renal hypotension wa accomplished by a screw clamp placed on shunt co connecting the right iliac artery and the renal arteries. Renal inflow pressure was measured from T-t the shunt distal t the clamp.

Regional cerebral hypotension was accomplished in the following ways: *A* In experiments, sh connected the left iliac artery and both vertebral arteries, which were dissected free and cannulated above the thoracic aperture. Another shunt connected the right iliac artery nd both common car

Table 1. Effects on mean systemic arterial blood pressure (AP), heart rate (HR), arterial plasma osmolality and arterial plasma glucose concentration evoked by interference with cardiovascular receptors and by regional tissue hypotension. Reported changes refer to maximal effects in individual experiments and to the interindividual range.

Experimental intervention		Increase of AP (mmHg)	Increase of HR (beats/min)	Increase of osmolality (mOsm/kg H_2O)	Increase of glucose (mM/L)
Bilateral vagotomy	5	unchanged	unchanged	1–4	0–3
Stimulation of carotid baroreceptors	5	5–20	0–10	1 5	1–4
Complete unloading of carotid baroreceptors	9	30–135	20–110	8–14	5–12
Liver hypotension 50 mmHg	3	unchanged	unchanged	unchanged	unchanged
Intestinal hypotension 50 mmHg	3	unchanged	unchanged	unchanged	unchanged
Renal hypotension 50 mmHg	3	unchanged	unchanged	unchanged	unchanged
Cerebral hypotension 50 mmHg	4	unchanged	unchanged	unchanged	unchanged

arteries. The sinus nerves were cut and about 10 min later the vertebral and carotid blood pressure was reduced down to 30–60 mmHg by adjusting screw clamps placed on each artery. In 2 expts. only the vertebral clamp was made, whereas the carotid arteries and sinus nerves were left intact. Cerebral hypotension to 50 mmHg was achieved by adjustments of the screw clamp on the vertebral artery.

Hemorrhagic hypotension was accomplished in some experiments by bleeding the animal into a pressure bottle connected via a T-tube to the right brachial artery. The exsanguination was rapid so that the arterial blood pressure reached the level of 50 mmHg within 3 min; this pressure level was then maintained during 30 min with the aid of the pressure bottle.

Blood pressure, heart rate and blood flow were recorded on a Grass Polygraph. Statham P23 AC transducers were used for the pressure measurements. Arterial plasma osmolality was determined with thermistor cryoscopy (Osmometer 3I LAS, Advanced Instruments, Inc.) and arterial plasma glucose concentration with the glucose-oxidase technique.

In Results section data are given as mean values ± S.E.

Results

In the control period before receptor interference, mean arterial blood pressure (AP) averaged 130 ± 5 mmHg, heart rate (HR) 185 ± 4 beats/min, arterial plasma osmolality 320 ± 2 mOsm/kg H_2O and arterial plasma glucose concentration 10.3 ± 0.7 mM/L.

A survey of the effects caused by interference with the activity of different receptors on AP, HR, and on arterial plasma glucose concentration and osmolality is given in Table 1. The results are described in some detail below.

Effects evoked from the carotid chemo- and baroreceptors

Bilateral cervical vagotomy was made about 30 min before interference with the carotid receptor activity. The vagotomy *per se*, leading to elimination of the afferent discharge from the aortic, cardiac and pulmonary mechanoreceptors, increased the arterial plasma glucose concentration and osmolality slightly, whereas AP and HR, except for an initial transient decrease, remained essentially constant.

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Renal hypotension (50 mmHg)	3	unchanged	unchanged	unchanged	unchanged
Cerebral hypotension (50 mmHg)	4	unchanged	unchanged	unchanged	unchanged

…teries. The sinus nerves were cut and about 10 min later the vertebral and carotid blood pressure was lowered down to 50–60 mmHg by adjusting screw clamps placed on each shunt. B. In 2 expts. only the vertebral shunt was made, whereas the carotid arteries and sinus nerves were left intact. Cerebral hypotension to 50 mmHg was achieved by adjustments of the screw clamp on the vertebral shunt.

Hemorrhagic hypotension was accomplished in some experiments by bleeding the animal into a elevated pressure bottle connected via a T-tube to the right brachial artery. The exsanguination was timed so that the arterial blood pressure reached the level of 50 mmHg within 3 min, this pressure level was then maintained during 30 min with the aid of the pressure bottle.

Blood pressure, heart rate and blood flow were recorded on a Grass Polygraph. Statham P23 AC transducers were used for the pressure measurements. Arterial plasma osmolality was determined with the freezing-point cryoscopy (Osmometer 31 LAS, Advanced Instruments, Inc.) and arterial plasma glucose concentration with the glucose-oxidase technique.

In Results section data are given as mean values ± S.E.

Results

In the control period before receptor interference, mean arterial blood pressure (AP) averaged 130 ± 5 mmHg, heart rate (HR) 185 ± 4 beats/min, arterial plasma osmolality 320 ± 2 mOsm/kg H_2O and arterial plasma glucose concentration 10.3 ± 0.7 mM/L.

A survey of the effects caused by interference with the activity of different receptors on AP, HR, and on arterial plasma glucose concentration and osmolality is given in Table I. The results are described in some detail below.

Effects evoked from the carotid chemo- and baroreceptors

Bilateral cervical vagotomy was made about 30 min before interference with the carotid receptor activity. The vagotomy *per se*, leading to elimination of the afferent discharge from the aortic, cardiac and pulmonary mechanoreceptors, increased the arterial plasma glucose concentration and osmolality slightly whereas AP and HR, except for an initial transient decrease, remained essentially constant.

indirectly by adrenergic control of catecholamine and glucagon release from the ad and pancreas (Järhult 1975 a)

The direct adrenergic effects on the heart and the blood vessels in hemorrhage are k to be initiated from several receptor areas in the circulatory system (e.g. Heymar Neil 1958 Öberg 1964 Chien 1967) but the receptors mediating the metabolic l glycemic response to bleeding have so far not been identified. An attempt was m the present investigation to analyse the possible role of certain cardiovascular recept this hyperglycemic response to hemorrhage

Methods

30 cats (mean b wt. 3.2 kg) were used in the study The animals fasted 12–14 h before the experim received water *ad libitum. They were anesthetized i with chloralose (50 mg/kg) and urethane (* mg/kg) after induction with ether A tracheal ca nula was inserted and the animal breathed spontar throughout the experiment. Heparin (1 000 IU/kg b wt.) was given prior to intravascular cannulatic *brachial artery was cannulated for recording systemic arterial pressure and for sampl g of arterial* Heart rate was recorded with a Grass tachograph triggered by the systolic pressure wave Muscle flow was estimated in some of the experiments by measuring with a optical drop recorder the out *blood flow from the cannulated right femoral vein In these expts. the right paw was remov* external pressure applied to the thigh so that flow through venous vessels other than the can *femoral vein was prevented without interfering with the arterial inflow*

In an attempt to reveal the possible role of various receptors in the circulatory system in the glycemic (hyperosmolar) response to hemorrhage, arterial plasma glucose concentration and osm were f llowed in the normotensive animal during alterations of receptor activity resembling qualit those occurring in bleeding. Fo this purpose, the carotid chemoreceptors were stimulated and the bar receptors unloaded in cats after bilateral cervical vagotomy The effects of vagotomy *per se* studied. The possible influence on plasma glucose concentration and osmolality from postulated me receptor sites in the renal, splanchnic and cerebral circulations was investigated as well (see Discu

The carotid chemoreceptors were stimulated according to the technique described by Little and (1975). The right iliac artery and vein were cannulated with polyethylene tubings and the arteri venous flows diverted through two separat channels of Harvard peristaltic pump. Distal to pum *arterial and venous tubings joined to a common tubing which after bifurcation was connected t* common carotid rteries. All mai rterial vessels in the vicinity of the carotid body were then l except for the external carotid arteries which were can ulated and the outflows diverted to a funnel *right external j gular vein. The carotid intra-si usal) pressure was recorded* bilaterally via T-tubes external carotid arteries and co ld be co trolled by means of screw clamps placed distal to the T With this technique for artificial flow perfusion, the chemoreceptors in the isolated carotid sinus regions could be stimulated by switch ng from arterial t venous blood perfusion under circumstan constant, normal pressure in the carotid si us. The normotension was accomplished by adjustments peristaltic pump and the screw clamps.

The carotid baroreceptors were inactivated in vagotomized cat with two d fferent techniques. (1) A plete unloading of the baroreceptors was simulated by cutting the sinus nerves bilaterally in the oth i tact sinus regions. (?) The above described preparation for rtificial perfusion of the carotid sinus r was used fo graded inactivation of the baroreceptors. For this purpose, the sinus regions were pe with arterial blood at mea *inflow pressures f bout 120, 90, nd 60 mmHg, respectively*

Local splanch ic hypotension was produced with an adjustable screw clamp placed on the coel superior mesenteric artery Regional blood pressure was recorded the left gastric rtery or in a *jejunal artery Local renal hypotensio* was accomplished by a screw clamp placed on a hunt connecting the right iliac artery and the renal arteries. *Renal inflow pressure* was measured from a T-t the shunt distal to the clamp.

Regional cerebral hypotension was accomplished in the following ways *A 1 2* experiments, connected the left iliac artery and both vertebral arteries, which were dissected free nd cannulate above the thoracic aperture. Another shunt connected the right iliac rtery and both common c

...LE II Effects on mean systemic arterial blood pressure (AP), heart rate (HR), arterial plasma glucose concentration, and arterial plasma osmolality evoked by stepwise reduction of the isolated carotid sinus pressure from about 120 mmHg dow to 90 and 60 mmHg, respectively The animals ere vagotomized in the neck about 30 min before experimental intervention. Data are expressed as the mean values from 3 expts

	Carotid sinus pressure ~ 120 mmHg	Carotid sinus pressure ~ 90 mmHg			Carotid sinus pressure ~ 60 mmHg		
		3 min	6 min	10 min	3 min	6 min	10 min
(mmHg)	124	176	155	147	185	138	118
R (beats/min)	198	220	217	221	231	233	235
ucose (mM/L)	12.8	14.6	15.3	15.5	16.8	18.5	18.9
molality mOsm/kg H_2O)	321	324	325	324	327	328	330

scular resistance in the lower leg. Arterial plasma osmolality also rose promptly within min it reached level about 9 mOsm/kg H_2O above control and this level was then roughly maintained during the rest of the observation period. The arterial glucose concentration creased rapidly to a steady level about 8 mM/L above control and thus accounted for the ajor part of the hyperosmolar response.

In 3 expts., the carotid baroreceptors were unloaded by stepwise reduction of the mean intrasinusal pressure from a control level of about 120 mmHg down to 90 and then to 0 mmHg. Table II shows that AP HR, arterial plasma glucose concentration and osmolality anged clearly and in a graded manner in response to these different levels of baroreceptor tivity

30 min after the sinus nerves were cut in the above described experiments (Fig. 1), the animals ere rapidly bled to an AP level of 50 mmHg maintained for another 30 min. On n average, 35% of the blood volume was shed after 30 min of hypotension and at that time here had occurred further increase of the plasma hyperosmolality by about 12 Osm/kg H_2O and of glucose concentration by about 9 mM/L. This increment of the smolality and glucose concentration can partly be explained by the decrease in blood olume (see Discussion), but the finding might also suggest that receptors other than hose firing in the agus and sinus nerves are involved in the hyperglycemic (hyperosmolar) esponse to hemorrhage. The possibility was approached in the experiments described elow

Effects of regional hypotension

Splanchnic and renal hypotension. Lowering of the arterial inflow pressure to 50 mmHg in the liver and intestine, or in the kidneys, did not cause any significant changes of the arterial plasma osmolality or glucose concentration, nor did it affect AP HR or lower leg blood flow

Cerebral hypotension. Reduction of the cerebral arterial inflow pressure to 50–60 mmHg with the two different techniques outlined in Methods did not cause any significant changes of AP HR, plasma osmolality or glucose concentration.

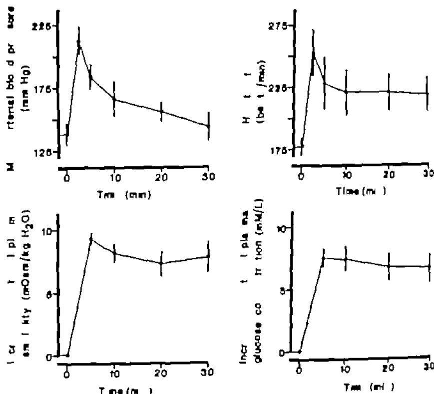


Fig. 1 Changes of mean arterial blood pressure, heart rate, arterial plasma osmolality and arterial pla
glucose concentration in response to bilateral cutting of the sinus nerves. The animals were vagotomi
in the neck about 30 min before the sinus nerve section. Mean values ±S.E. are given

Stimulation of chemoreceptors These receptors were stimulated by shifting from arteri
to venous blood perfusion of the sinus-glomus regions, maintaining the intra-sinusal me
pressure at a normal level. The induced decrease in pH and pO and the increase in pC
during venous perfusion will here determine the extent of receptor activation. Duri
arterial perfusion pO averaged 10 6 kPa, pCO 3 8 kPa, and pH 7.38. During veno
perfusion the corresponding values were 5.2 kPa, 6.1 kPa and 7 24 which are figures simi
to those occurring during severe hypovolemia (*e g* Schweizer and Howland 1972). Such
chemoreceptor stimulation caused a moderate increase in AP as well as in skeletal mus
vascular resistance, whereas HR remained unchanged or increased slightly (in accordan
with Little and Öberg 1975). A small but invariable increase was seen in the arterial plas
glucose concentration and osmolality

Unloading of baroreceptors A selective and complete elimination of the dischar
from the carotid baroreceptors, without introducing an artificial perfusion system, w
achieved by sectioning of the sinus nerves in the vagotomized cat Fig. 1 shows the chang
of AP and HR and the increase of arterial plasma osmolality and glucose concentratio
during an observation period of 30 min after section of the sinus nerves. HR increas
markedly to attain a steady level about 50 beats/min above control AP rapidly reached
peak increase about 80 mmHg above control, after which it gradually declined towards t
control level. The changes of HR and AP were associated with a clear-cut increase of t

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Discussion

The present study shows that unloading of the carotid baroreceptors evokes a m increase in the arterial plasma glucose concentration with an attendant increase i arterial plasma osmolality Preliminary experiments indicate that such a response c evoked also from the aortic baroreceptor region The carotid and aortic baroreceptors can initiate quite a drastic "metabolic" adjustment besides the important reflex infl on the heart and the blood vessels.

Numerous studies have demonstrated that hemorrhage is associated with hypergly (for ref see Järhult 1975 b) This investigation seems to indicate that the hemorrhagic h glycemia to a large extent is initiated from unloading of the carotid arterial barorecep but the quantitative importance of these receptors in the response is difficult to eva exactly from the present data. A direct comparison between the magnitude of hypergly evoked by complete elimination of the baroreceptor discharge in normovolemic an and that seen after bleeding to 50 mmHg (see Results and Järhult *et al* 1976) suggests about 50 per cent of the latter response would be due to the baroreceptor reflex. It sh be pointed out however that the plasma concentration of course increases much during hypovolemia than during normovolemia, simply because of the much smaller volume available for glucose dilution in the former situation. Furthermore, the m decrease of blood flow through most tissues during hemorrhage implies that a signific lower capillary transfer of glucose from the intra to the extravascular space will occ bleeding than during baroreceptor inactivation in the resting normovolemic animal considerations indicate that the baroreceptor reflex is the main mediator of the hemorr hyperglycemia response It is quite possible however that other receptor stations also con ute to this metabolic adjustments. The present results thus suggest that the carotid ch receptors are of some importance while baroreceptors in the renal circulation as w postulated baroreceptors in the splanchnic (*e.g* Tuttle and McCleary 1975) and cer (*e.g* Hume 1974) circulation do not contribute to the initiation of hemorrhagic hypergly

The efferent pathways in the hemorrhagic hyperglycemia response involve several of the sympatho-adrenal system, viz. direct sympathetic fibres to the liver relea catecholamines from the adrenal glands, adrenergic release of glucagon from the pan (Järhult 1975 a) and adrenergic inhibition of the insulin output (Carey *et al* 1970, H *et al* 1973). It seems likely that the reflex baroreceptor hyperglycemia makes use of the adrenergic mechanisms an assumption supported by some preliminary experiments w demonstrate that elimination of the carotid baroreceptor discharge leads to a pro increase in the glucagon and decrease in the insulin concentration (Järhult and Holst 19 In the CNS *the ventromedial part of the hypothalamus may possibly serve to integrate* reflex, since stimulation of this structure has been shown to cause hyperglycemia increased plasma levels of adrenaline noradrenaline and glucagon (Dunér 1953 Fol and Euler 1954 Frohman and Bernardis 1971).

This study was supported by a gra t from the S edish Medical Research Council (B76-14X 2210- and from the Faculty of Medicine, U iversity of Lund.

ing membrane of the cat) and by Gibson and Pollock (1973 anococcygeus muscle of rat). I both cases, the potassium induced contractures were reduced not only by renoceptor blockade but also by denervation.

e present experiments studied in more detail the influence of intramural noradrenaline e potassium induced contracture in the uterus and in the portal vein of the rat. These organs have different adrenergic responses, noradrenaline has a relaxant effect on the is (Schild 1967) whereas it activates the rat portal vein (Johansson *et al.* 1967). eliminary results suggested that the potassium induced release of noradrenaline depended he estrogenic influence on the smooth muscle. This point is further analyzed here.

Material and Methods

als Uterine muscle and portal vein of 60 rgan Wistar rats weighing 130–200 g were used. All rats oophorectomized. 29 of them were given 0.5 mg of polyestradiol phosphate i.m. immediately after tion (This produces an almost constant estrogen effect for several weeks in spayed mice — Diczfalusy 1954) These rats were killed on day 4 after operation. The other 31 animals did not receive none treatment and ere killed on day 7 after oophorectomy

nimals from each group were given an p. injection of reserpine, 5 mg/kg, 30 h before the expt. In rs, this treatment depletes the uterus of catecholamines within 24–36 h (Owman and Sjöberg 1967). *paration and mounting* The uterus as removed and placed in Krebs solution (composition, see w) The lumens of the uterine horns were split open by longitudinal section at the mesenteric border e organ Strips of the uterus, about 15 mm long, ere mounted vertically in 30 ml jacketed organ s containing Krebs solution The preparations were initially subjected to tension of 10 mN Within nin, the preparations relaxed to tension level of 1 2 mN which as maintained during the expt. r stabilizing period of 30 min, during hich spontaneous activity developed, the bathing fluid as nged to an isotonic potassium solution (composition, see below).

he portal vein as exposed and removed together with some surrounding tissue, placed in Krebs tion, and then dissected free. Ligatures were put around the ein at the point where joins the superior enteric vein and at the hepatic end, and the lumen was opened longitudinally The preparations ere nted vertically as described for the uterine strips Initial tension was adjusted to approximately 2.5 mN, hich the length of the ein between the ligatures as about 10 mm. After 30 min, the bathing fluid was nged to the isotonic potassium solution. I some expts., repeated contractures were produced. The e of exposure to the potassium-rich solution and also the recovery period (in Krebs solution) between ntractures ere kept constant at 15 and 30 min, respectively

The contractile responses were recorded isometrically by tension transducer (Grass FT-03-C) connected polygraph ink-writer (Grass P7 or Beckman R611).

Measurement of responses Results obtained in expts. on the rat uterus are principally demonstrated as ginal records, whereas results from expts. on the rat portal vein are given as average responses. For the ter purpose, the maximum amplitude of each individual preparation during potassium contracture s set at 100 and served as reference. During the first 3 min of contracture, the tension was measured 0 5-min intervals and expressed as per cent of the maximum of the response. Normalized values from ferent preparations were pooled, and mean alues ere calculated at each respective time

The results showing effects of repeated stimulation are given as the integrated contractile activity during e first 10 min of the contracture The contraction record was copied on photographic paper and the area der the myogram was cut out and eighed. The first response in series of consecutive contractures as ually the largest I as chosen as the reference and set at 100 . All other responses in the same expt. were pressed in per cent of this maximum contraction.

Statistical significance was determined by Student' t-test.

Solutions The muscle strips ere bathed in Krebs solution of the following composition (mM): NaCl 119 KCl 4 6, $NaHCO_3$ 20, $CaCl_2$ 1 5, NaH_2PO_4 1.2, $MgCl_2$ 1.2, glucose 11, pH 7 4. The contracture olution had the following composition (mM): KCl 127 $NaHCO_3$ 20, $CaCl_2$ 1.5, NaH_2PO_4 1.2, $MgCl_2$ 1.2, glucose 11 pH 7.4 The solutions were prepared on the day of the expt. Double-distilled water and analytical grade chemicals were used

Acta physiol scand. 1977 101 112-121
From the Department of Pharmacology University of Lund, Sweden, and the Institute of P
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The Role of Intramural Noradrenaline in the Potassium Induce Contracture of Non Estrogenized Smooth Muscle

By

BENGT BENGTSSON

Received 31 March 1977

Abstract

BENGTSSON B. *The role of intramural noradrenaline in the potassium induced contract non-estrogenized smooth muscle* Acta physiol scand. 1977 *101* 112-121

The influence of i tramural noradrenaline on potassium induced contractures was studied in n preparations of the uterus and the portal vein of the rat. Uterine strips of oophorectomized rats resp with contraction followed by transient relaxation when immersed in med um containing 127 mM al m chloride. Reserpinization or blockade of β-adrenoceptors with propranolol greatly diminish transient relaxation. In the isolated portal vein both noradrenaline depletion with reserpine and α-ad ceptor blockade (phentolamine) reduced the active tension produced i response t the isotonic pot solution. These results suggest that intramural noradrenal ne plays a significant role in the develop of contractures evoked by potassium in the uterus and the portal vein of the rat. In the uterus, the rel noradrenaline counteracts the development of active tension, whereas in the portal ein, noradre has a contractile effect which is added to that of potassium. Estrogen treatment reduced (portal e abolished (rat uterus) the contractile effects of intramural noradrenaline. The adrenolytic effect of est is possibly due to reduction in the release of noradrenaline from the sympathetic nerve-endings.

Potassium in high concentrations depolarizes the cell membrane and initiates smooth m contraction. However it has also been shown that in high concentration it causes a relea noradrenaline from adrenergic nerve-endings (Kirpekar and Wakade 1968), and i reasonable to presume that released noradrenaline should modify the potassium indu contractures in adrenergically innervated smooth muscle (Furchgott 1955) In suppor this idea, several reports describe an inhibitory effect of α adrenoceptor blocking agent potassium contractures in vascular smooth muscle (Bevan *et al* 1963 Shibata and Car 1967 Somlyo and Somlyo 1969 Somlyo *et al* 1969 Peiper *et al* 1971 Golenhofen *et* 1973 Massingham 1973) However these effects have not generally been attributed inhibition of released noradrenaline, but rather to a direct inhibitory effect on membrane permeability to activating cations (Bevan *et al* 1963 Shibata and Carrier 19 Somlyo and Somlyo 1969 Somlyo *et al* 1969)

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The Role of Intramural Noradrenaline in the Potassium Induced Contracture of Non-Estrogenized Smooth Muscle

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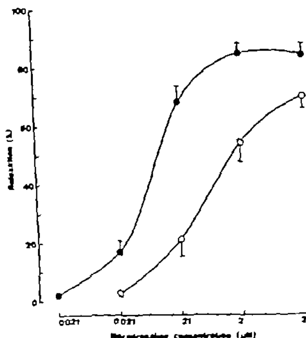
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Potassium in high concentrations depolarizes the cell membrane and initiates smooth muscle contraction However it has also been shown that in high concentration it causes a release of noradrenaline from adrenergic nerve-endings (Kirpekar and Wakade 1968) and it is reasonable to presume that released noradrenaline should modify the potassium induced contractures in adrenergically innervated smooth muscle (Furchgott 1955). In support of this idea, several reports describe an inhibitory effect of α-adrenoceptor blocking agents on potassium contractures in vascular smooth muscle (Bevan *et al* 1963 Shibata and Carrier 1967 Somlyo and Somlyo 1969 Somlyo *et al* 1969 Peiper *et al* 1971 Golenhofen *et al* 1973 Massingham 1973). However these effects have not generally been attributed to inhibition of released noradrenaline but rather to a direct inhibitory effect on the membrane permeability to activating cations (Bevan *et al* 1963 Shibata and Carrier 1967 Somlyo and Somlyo 1969 Somlyo *et al* 1969).

More substantial evidence in favour of the idea that potassium stimulates smooth muscle indirectly through release of noradrenaline has been produced by Cervoni (196



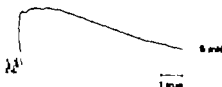
...e relaxant effect of nor... on rat myometrium, ...d by 3 mM calcium in ... potassium solution, ex... as percentages of con... traction. Solid circles: ... Open circles: estrogenized ... Each point represents the ... ± S.E. of 6 expts.

b) or addition of 2 · 10^{-6} M propranolol (Fig. 2 c) or 10^{-6} M atropine to the bathing n (Fig. 2 d) had no detectable effects on the potassium induced contracture when imals had been estrogenized. The spontaneous contractile activity was sparse and ar in the myometrium of estrogenized rats and did not change appreciably after lalization or addition of propranolol.

Effect of noradrenaline on the depolarized uterus. Fig. 3 shows the relaxant effect of renaline added to a calcium induced contracture of the depolarized rat uterus. In the strogenized tissue, maximum relaxant effect was attained at approximately 2 µM renaline. Estrogen treatment caused a shift of the log concentration–response curve right, increasing the EC_{50} value by about 10 times.

rtal vein

Nephrectomized rats without estrogen substitution. Fig. 4 illustrates a typical contractile ...se of an isolated strip of rat portal vein during immersion in isotonic potassium ...ion. The tension immediately rises to about maximum, is maintained for about .. min, then declines slowly



4. Contractile response of rat portal vein (non-...genized) induced by immersion in isotonic po-...ium solution.



Fig. 1 Spontaneous contractions followed by potassium induced contractures of isolated rat uterus control, (b) after reserpinization, (c) in the presence of 2 10^{-6} M propranolol, (d) in the presence PGE$_1$ (6 × 10^{-} M).

The bathing solution was kept at 37°C and aerated with a mixture of 5% CO_2 and 95 O_2.

Drugs Atropine (BDH), noradrenaline bitartrate (Apoteksbolaget, Sweden), phentolamine (Regitine Ciba), polyestradiol phosphate (Estradurin® LEO), propranolol (Inderal ICI Pharma), prostaglandin E$_1$ (Astra) and reserpin (Serpasil® Ciba) were used in the expts.

The drugs were injected into the bath in volumes of 0.03–0.1 ml, 10 min before changing the K solution to the potassium-rich medium.

Results

I *Uterus*

A *Oophorectomized rats without estrogen substitution* The rat uterus responds with contraction when depolarized by potassium. Fig. 1 a shows a typical potassium contracture in uterine strip from an oophorectomized rat As can be seen there is an initial contraction followed by a transient relaxation and a slow rise in tension.

Reserpinization greatly diminished the transient relaxation that normally occurs during the first few minutes in a potassium rich solution (Fig. 1 b). The preceding spontaneous contractions were not obviously different after reserpinization.

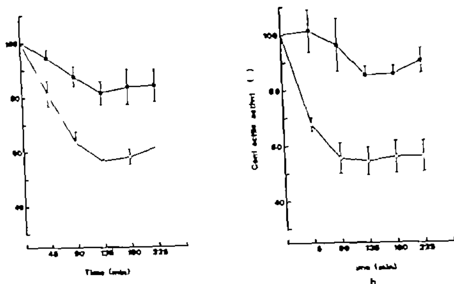
Blockade of the β-adrenoceptors in the myometrium with propranolol (2 10^{-6} M) affected the potassium contracture in the same way as reserpinization. Thus, the drug reduced the transient relaxation and caused a better maintained active tension (Fig. 1c). There was no observable change in the spontaneous contractions in the presence of propranolol.

Prostaglandin E (PGE$_1$) increased the amplitude of the spontaneous contractions but did not remove the transient relaxation in the depolarized myometrium (Fig. 1 d). (Similar results were obtained with PGE$_2$).

B *Oophorectomized rats treated with estrogen* Uterine strips from oophorectomized rats treated with polyestradiol phosphate exhibited no phasic contraction in response to isotonic potassium solution, but the tension continued to increase during the first 2 3 min. The tension thereafter remained unchanged or declined slowly Reserpinization of the anim



Fig. 2. Contractile responses of estrogenized rat uterus induced by immersion in a medium containing [illegible] mM potassium. ( ) control, (b) after reserpinization, (c) in the presence of 10^{-6} M propranolol, (d) in the presence of 10^{-6} M atropine.



7 a. Effect of repeated activation (127 mM potassium) on the contractile activity of the rat portal vein. trol (solid circles) and estrogenized (open circles) animals. Each point represents the mean value and S.E. of 4 expts.

7 b. Effect of repeated activation (127 mM potassium) on the contractile activity of the rat portal vein. trol (solid circles) and estrogenized (open circles) animals. Phentolamine (10^{-4} M) added to the bathing tions in both series. Each point represents the mean value and the S.E. of 4 expts.

B *Oophorectomized rats treated ith estrogen.* Fig. 6 shows the average response to tassium in estrogenized portal veins. There was a statistically significant reduction of muscle tension during the first 2 min of the contracture of the estrogenized preparation mpared with the control. Reserpinization caused a more pronounced relaxation in rogenized portal eins than in the non-estrogenized tissues (*cf* Fig. 5). The average ponse of essels treated with reserpine coincided with that obtained in the presence of entolamine (10^{-4} M).

C. *Effect of repeated activation by potassium.* When rat portal veins were subjected to peated depolarizations with the isotonic potassium solution, the contractile responses clined to a steady level which was reached after about 4 contractions, 135 min (Fig. 7 a). his decline in contractile response was 20% in the non-estrogenized preparations and 0 in preparations from estrogen treated animals.

The reduction of contractile response in rat portal veins during repeated depolarizations ould have been due to a gradual loss of noradrenaline from the intramural nerves. In order o test this, repeated depolarizations were performed in the presence of 10^{-4} M phentolamine Fig. 7 b). The percentage reduction of the contractile response was about the same as in he absence of α-adrenoceptor blockade (*cf* Fig. 7), although the absolute values were maller. Again, the responses declined more in preparations from estrogen-treated animals.

D *Effect of noradrenaline in the depolarized portal vein.* Fig. 8 shows the contractile effect of noradrenaline added to a calcium induced contracture of the depolarized rat portal vein.

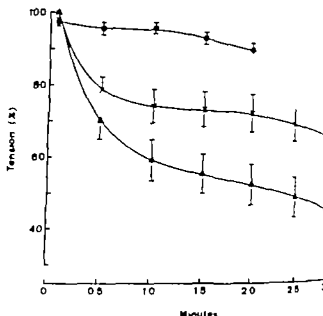


Fig. 5 Average time course of contractures (n=9) evoked by 127 mM potassium in isolated portal veins of non-estrogenized rats. Solid circles control. Crosses preparations from reserpinized animals. Triangles preparations from untreated animals with 10^{-4} M phentolamine added to the bathing solution.

Fig. 5 shows the influence of reserpinization and α-adrenoceptor blockade on the time course of potassium contractures in the isolated portal vein. After reserpine treatment there was a more rapid relaxation. 1 min after initiation of the response, the contractile force of the reserpinized preparations had decreased by about 25%. Similar but more pronounced effects were produced by phentolamine (10^{-4} M). The muscular tension decreased by 40% during the first minute of potassium contractures evoked in the presence of phentolamine

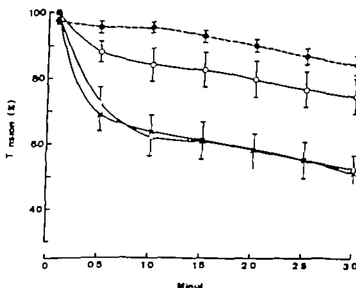


Fig. 6. Average time course of contractures (n=8) evoked by 127 mM potassium in isolated portal veins of the rat. Solid circles (broken line) control (no estrogen treatment), same data as in Fig. 5. Open circles estrogenized preparations. Crosses: preparations from estrogen-treated and reserpinized animals. Triangles preparations from estrogenized animals with 10^{-4} M phentolamine added to the bathing solution. Asterisks indicate that the difference between values of control and estrogenized preparations is statistically significant ($p < 0.05$).

rer the expts. suggest that reduced sensitivity of the myometrium to noradrenaline not account quantitatively for the finding that estrogen treatment removes the rgic influence on the potassium induced contracture. A dose of noradrenaline that maximum (80 %) relaxation in the non-estrogenized uterus can be shown to give at 0 relaxation in the estrogenized muscle (Fig. 3). The complete absence of relaxation estrogenized uterus therefore suggests that an *insufficient amount* of noradrenaline leased to act on the myometrium in the latter case.

Ier studies of the effects of estrogen on the noradrenaline contents of the rat uterus led to different conclusions. Histochemical data suggest that estrogen treatment ses the fluorescence of adrenergic nerves in rat myometrium (Hervonen *et al.* 1973 nova *et al.* 1974), whereas biochemical studies indicate that the total amount of renaline in the rat uterus is independent of estrogen (Oskarsson 1960, Falck *et al.* 1974). e uterine weight is increased by estrogen, the latter results suggest that the quantity radrenaline per g of uterine tissue is reduced by estrogen treatment. However even *derate decrease in the relative quantity of noradrenaline in the estrogenized rat uterus* d not explain the complete absence of relaxant effect of intramural noradrenaline after gen treatment, as shown by the present results. It is possible that estrogen inhibits the e of noradrenaline from adrenergic nerve endings.

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o judge from the present results, the sensitivity of the α-adrenoceptors of the rat portal is not appreciably changed by estrogenization. This suggests that estrogen inhibits adrenergic influence in the rat portal vein by reducing the amount of noradrenaline t acts on the receptors.

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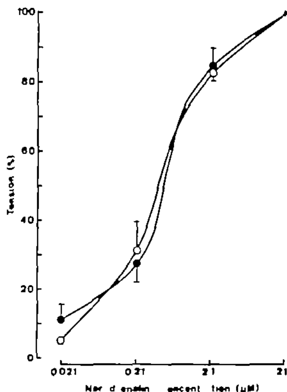


Fig. 8 The contractile effect of noradrenaline rat portal veins immersed i isotonic potas solution containing 3 mM calcium Solid ci control. Open circles, estrogenized tissue. point represents the mean and the SE expts.

Maximum contractile effect was attained at a noradrenaline concentration of about 10^{-1} Estrogen treatment did not change the sensitivity of the rat portal vein to added noradr aline

Discussion

The present study showed that the mechanical response of the rat uterus and portal to a potassium-rich medium might be modified by simultaneously released noradren The evidence in favour of this seems fairly conclusive as blockade of the adrenocep resulted in changes almost identical to those induced by noradrenaline depletion reserpine.

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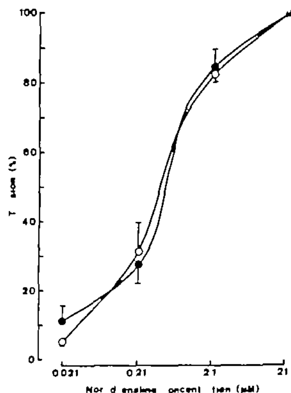


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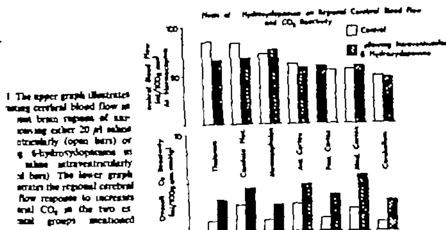
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1 The upper graph illustrates resting cerebral blood flow in rent brain regions of receiving either 20 µl saline intraventricularly (open bars) or µg 6-hydroxydopamine in saline intraventricularly (black bars). The lower graph strates the regional cerebral flow response to increases arterial CO_2 in the two experimental groups mentioned

bility by testing the cerebral CO responsiveness following the intraventricular injection hydroxytryptamine, an agent which is selectively neurotoxic for the catecholamine-aining neurones (Malmfors and Sachs 1968).

experiments were carried out in 23 adult male Sprague-Da ley rats (300 g, b t), fed and watered *ad* The rats ere divided into 4 groups. The first tw groups received an intraventricular injection of saline (20 µl) one of these groups was studied at normocapnia (35–40 mmHg, 5 rats), the other t capnia (50–65 mmHg, 7 animals). The last tw groups of rats received an injection of 250 µg 6-hydroxy-amine (6-OHDA-HCl in 20 µl saline) into the lateral entricle and were again studied at either normo-a (4 rats) or hypercapnia (6 rats). The intraventricular injections were given stereotaxically under light ether anaesthesia (Brietal, Lilly 40 mg/kg) 7 days prior to the blood flow measurements. Various of the brains were processed for fluorescence histochemistry according to the Falck Hillarp for-aldehyde method (for technical details see Björklund, Falck and Owman 1972).

Anesthesia was induced ith 2 3 halothane in O_2. Following cannulation of the trachea, the animals ventilated artificially and anesthesia as maintained with 70 N_2O- 30 O_2 gas mixture. Body perature as kept constant at 37°. Cerebral blood flow was measured by the [14]C-ethanol technique, eloped by Eklöf *et al* (1974). The femoral arteries and veins were cannulated on both sides. One of the rial catheters was used for the recording of mean arterial pressure, the other was used for the measure- of arterial pCO_2, pO_2, pH and radioactive ethanol concentrations. One enous catheter was used to se the tracer ([14]C-ethanol, 20 mCi in saline) whilst the other catheter as used to inject saturated KCl he termination of the blood flow estimation.

The radioactive tracer was infused over 30 s, arterial samples (30 µl) being withdrawn every third second l the animal was sacrificed as soon as the last sample had been obtained. The rat as decapitated and the d was immediately frozen in liquid nitrogen. At a later stage, seven brain regions (see Fig 1) were ected out, each piece of tissue being dissolved th 0.5 ml Soluene (hich 10 ml Instagel was later ded. The radioactivity in tissue and arterial samples was assessed in standard liquid scintillation counter d quench correction as performed according to established principles.

In each brain region, cerebral blood flow was plotted against arterial CO_2 and a standard near regression was calculated using the least squares method. The slopes of these regres- ons yielded the cerebrovascular CO_2 reactivity (in ml/100 g min.mmHg) for each brain egion in saline-treated and in 6-OHDA-treated rats. Statistical difference between overall regressions was studied by an analysis of variance.

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Effects of Intraventricular 6-Hydroxydopamine on Cerebrovascular CO_2 Reactivity in Anesthetized Rats

By

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Abstract

EDVINSSON L., J E. HARDEBO and E. T MACKENZIE. *Effects of intraventricular 6-hydroxydopamine on cerebrovascular CO reactivity in anesthetized rats* Acta physiol. scand 1977 *101* 122-125

Regional cerebral blood flow was measured by the C-ethanol technique in anesthetized rats before and after intraventricular injection of 6-hydro ydopamine. This treatment reduced the fluorescence of central noradrenaline and dopamine nerve terminals, as well as of the perivascular nerve terminals of cerebral vessels. The dministration of 6-hydroxydopamine had no significant effect on cerebral blood flow at normocapnia. The cerebrovascular reactivity to hypercapnia was sign ficantly increased in the 6-hydroxydopamine treated animals. The results indicat an involvement of central catecholamine pathways in the cerebrovascula reactivity to hypercapnia.

The most widely recognized and documented responses of the cerebral circulation are the increase in cerebral blood flow secondary to hypercapnia, and the decrease in cerebral blood flow secondary to hypocapnia Although the exact mechanism of action of arterial CO_2 is unknown, it is believed generally that this action involves pH changes in the ionic milieu that surrounds cerebral resistance vessels (for review see Lassen 1974) However one series of experiments would speak against this hypothesis a number of studies have shown that pontine transections, or ablations can severly attenuate or even completely abolish the cerebral vasodilatatory response to induced hypercapnia (Capon 1975 Fenske *et al* Shalit *et al* 1967) The intracarotid infusion of the β-adrenolytic agent, propranolol similarly reduces the cerebral circulatory reactivity to induced hypercapnia (MacKenzie, McCulloch and Harper 1976)

These observations might suggest that the central catecholaminergic systems—which arise from the brain stem—are in some way involved in the cerebrovascular reactivity to changes in arterial CO_2 tension The purpose of the present investigation was to examine

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Fluorescence histochemistry In those animals treated with 6-OHDA, demonstr marked overall reduction of the central noradrenaline (NA) and dopamine (DA) terminals Very few NA and DA nerve terminals remain within the zone 2 mm fro ventricular surface terminals outside this zone were also clearly reduced. Whole bra and DA have been shown to be reduced to 34 and 46%, respectively of control 2 dr a single intraventricular injection of 250 μg 6-OHDA (Iversen and Uretsky 1971). A n depletion of the perivascular nerve terminals in the cerebral vessels was noted in the p investigation

The intraventricular administration of 6-OHDA had no significant effect on c blood flow at normocapnia ($P > 0.05$ in every brain region Student's t-test for un data) However the CO reactivity—the slope of the relationship between cerebral flow and arterial CO_2 tension—was significantly increased ($P < 0.01$ analysis of v between overall regressions)

Although the findings of this investigation indicate that the neurotoxic effects of 6-(to the central catecholaminergic pathways may affect cerebrovascular CO_2 reacti interpretation of the physiological significance of these findings is difficult Firstly it be recognized that intraventricular 6-OHDA destroys dopaminergic as well as noradr neurones (Iversen and Uretsky 1971 Ungerstedt 1971 a) Secondly the intravent administration of 6-OHDA is neurotoxic to the perivascular (sympathetic) nerves, in a to the central catecholaminergic neurones. This was shown in the present investi Nonetheless, it is unlikely that the perivascular sympathectomy contributed materi the observed response as a number of investigations have shown that neither cervica pathectomy nor α-adrenergic blockade will influence the cerebrovascular response to capnia (for review see Edvinsson and MacKenzie 1976) The third problem with the pretation of this preliminary report concerns the development of denervation hypersens As is the case with peripheral sympathetic nerves, the degeneration of central noradr neurones leads to a denervation hypersensitivity of the pre and postsynaptic recept is initiated approximately two days after the denervation the loss of presynaptic terminals is associated with a diminished reuptake of noradrenaline (Trendelenburg The injection of 6-OHDA into the lateral ventricle causes a specific degeneration of c catecholaminergic nerve terminals and a depletion of catecholamines (Iversen and U 1971 Ungerstedt 1971 a) A central denervation hypersensitivity has been associate the administration of 6-OHDA on a number of occasions (Huang and Daly 1974 U stedt 1971 b) It has been demonstrated recently that the intraventricular administrat 6-OHDA effects an increase in the density of β-receptors in the rat cerebral cortex, w affecting the affinity of the receptors for the β-adrenergic agonist, isoprenaline (et al 1976).

Future investigations into the relationship between the central catecholamine pat and the cerebral circulatory responsiveness to hypercapnia will need to examine s lesions of the noradrenergic or dopaminergic tracts at various intervals, following the ment of the lesions, before this relationship is well understood

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Acta physiol. scand. 1977 101 126–128

From the Minerva Institute for Medical Research, and
Research Laboratories of the State Alcohol Monopoly (Alko), Helsinki, Finland

Effects of Ethanol on Urinary Arginine Vasopressin Excretion in T Rat Strains Selected for Their Different Ethanol Preferences

By

JAAKKO LINKOLA, FREJ FYHRQUIST and OLOF FORSANDER

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Abstract

LINKOLA, J., F FYHRQUIST and O FORSANDER. *Effects of ethanol on urinary arginine va pressin excretion in two rat strains selected for their different ethanol preferences.* A physiol scand 1977 *101* 126–128

The effects of ethanol on urinary excretion of arginine vasopressin (AVP), sodium, and potassium investigated in two rat strains specially selected for their different alcohol preferences. The alcohol prefer (AA) strain excreted more AVP and the water preferring (ANA) strain more urine and sodium during hours after ethanol intubation (2 4 g/kg b.w 20 % v/v). The data is insufficient to establish a causal r tionship between differences in water and electrolyte metabolism and voluntary ethanol consumption.

Ethanol affects the water and electrolyte balance in man and laboratory animals (Wall and Barry 1970). Recently differences have been found in the diuretic behaviour and elect lytic excretion of two rat strains selected for their voluntary ethanol ingestion (Link 1976) At the same time, attempts to increase voluntary ethanol consumption of animals inducing changes in this balance have been unsuccessful Thus, it seemed important investigate more closely the hormonal regulation of water metabolism in the etha preferring AA (Alko Alcohol) strain and water-preferring ANA (Alko, Non-Alcoh strain (Eriksson 1968, Eriksson 1971)

Urinary excretion of arginine vasopressin (AVP) reflects the plasma concentration of t polypeptide hormone (Miller and Moses 1971) We therefore studied the urinary excret of immunoreactive AVP in AA and ANA rats after the administration of a single dose ethanol Urinary excretion of sodium and potassium was also recorded

Material and Methods

10 males and 10 females aged 3 months, were taken from each of AA and ANA strains (F_{ss} generat fo use in the expts. They had been given standard ASTRA EWOS (Södertälje, Sweden) rat food and water freely until the expt. began At 9 a.m. they were intubated with 2.4 g/kg ethanol as 20 % v/v solu in distilled water Urine was collected f 6 h as described earlier (Linkola 1976). The collecting tubes w kept in an ice bath.

I Urine output (UO) and osmolality (Osm), urine arginine vasopressin concentration (AVPc), urinary arginine vasopressin output (AVPo), sodium (Na) and potassium (K) output ± S.D. during 6 h after ethanol administration (2.4 g/kg b.w. 20% [illegible]) in AA and ANA rats. In both groups [illegible] 20, if not mentioned, p represents the significance of differences

UO ml/kg b.w.	Osm mOsm/kg	AVPc pg/ml	AVPo pg	Na mmol/kg b.w.	K mmol/kg b.w.
8.0 ± 3.9	1551 ± 742 n 17	145.1 ± 142.1 17	265.6 ± 152.2 n 17	0.4 ± 0.3	1.1 ± 0.4
p 0.001	p 0.001	p 0.005	p 0.05	p 0.001	p 0.10
18.5 ± 5.6	601 ± 200	36.6 ± 22.5	137.5 ± [illegible]	1.0 ± 0.5	1.2 ± 0.4

ary vasopressin concentration as measured by radioimmunoassay (F) Inquest et al 1976) modified assay of AVP in rat urine. The urine as diluted tenfold with distilled water to avoid non-specific ence. Blank tubes without antiserum yielded values close to zero. Recovery of added synthetic vasopressin (Sigma VI, 100 I.U./ml, St. Louis, Missouri) in the range 2.8–45.0 pg/ml as about 85 ... ecific interference as negligible when urine as diluted 1 10 or more. All samples from both rat were assayed at the same time. Urine sodium and potassium concentrations were determined by photometer (Eel), and the osmolality by vapor-pressure osmometer (Wescor).

Results

ry output of AVP was approximately 1.9 times higher in the AA strain (Table I). ifference in urine AVP concentrations was even more significant (p 0.005). *Urine* t was about 2.3 times greater in the ANA strain than in the AA strain, and urine lality about 2.6 times higher in the AA strain than in the ANA strain (Table I). Urinary m output was about 2.5 times higher in ANA than AA rats, but no significant difference bserved in the potassium excretion.

Discussion

hypothesis that *disturbances in the water and electrolyte balance may stimulate* oluntary ol drinking has been tested in animal expts. The results of Iida (1957) with mice do support the hypothesis, and Balmet and Montastruc (1962) even observed decrease ulatory ethanol consumption in dogs treated with antidiuretic hormone. Eriksson 7) showed that two diuretic drugs had no effect on alcohol consumption in rats.

recent report (Linkola 1976) revealed differences in the water and electrolyte metabolism rat strains selected by outbreeding *to drink, in a free-choice situation, as much ethanol* ssible (AA strain) or to avoid ethanol (ANA strain) (Eriksson 1971). Our findings here ate that the different diuretic behavior of AA and ANA rats can be explained by dif ces in the antidiuretic hormone release.

ntidiuretic hormone release is a consequence of complex events. Among these are the itivity of osmoreceptors and the hypothalamic neurones governing the release of vaso- sin. Functional differences in these structures may be responsible for strain differences vasopressin release.

The reason for higher sodium output in ANA rats than AA rats remains [illegible] It may be a direct effect of AVP (Finn 1973) or may indicate differences in other [illegible] controlling systems, located either at the central level (Dorn *et al* 1970) or in the [illegible] cortex (Knochel and White 1973)

It has been observed in rats (Marquis *et al* 1975) that ethanol causes only a [illegible] suppression of vasopressin release, followed by a rapid increase in AVP production. [illegible] we suggest that the stimulation of vasopressin release after ethanol administration [illegible] be emphasized when the effects of ethanol on water balance are being considered. [illegible] work is needed to investigate the relationships between osmoregulation and [illegible] ethanol drinking.

This study was supported by the Finnish Foundation for Alcohol Studies and the Sigrid Juselius [illegible]

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Acta physiol. scand. 1977 101 129–143

From the Department of Pharmacology University of Odense, Denmark

Neuronal and Extraneuronal Outflow of ^{3}H Noradrenaline Induced by Electrical-Field Stimulation of an Isolated Blood Vessel

By

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Abstract

Schrold J and O A. Nedergaard *Neuronal and extraneuronal outflow of ^{3}H-noradrenaline induced by electrical-field stimulation of an isolated blood vessel.* Acta physiol. scand. 1977 *101* 129–143

The adrenergic neuronal selectivity of ^{3}H-outflow elicited by electrical-field stimulation of rabbit isolated pulmonary artery preloaded with ^{3}H-(–)-noradrenaline (^{3}H-NA) was examined. Following incubation (30 min) of arterial rings at 37°C with ^{3}H-NA (10^{-6} M) and a 100 min wash-out period, the sympathetic neurones were stimulated selectively and supramaximally as regards tension development, with a constant current (250 mA, 300 monophasic pulses, 10 Hz, 0.3 ms). The initial stimulation-induced ^{3}H-outflow was greater than the subsequent 5 outflows, which remained almost constant (39, 20, 19, 17, 16 and 16 pmol g^{-1} respectively). ^{3}H-NA and ^{3}H-*O* methylated plus deaminated metabolites constituted 11 and 74 %, respectively, of the total ^{3}H-outflow induced during the initial stimulation period, but 38 and 38 %, respectively, during the second stimulation period. Omission of Ca^{++} in the physiological salt solution reduced the 6 stimulation-induced outflows to 67, 40, 35, 24, 21 and 21 %, respectively, of the untreated preparations. Bretylium (3×10^{-6} M) or tetrodotoxin (10^{-6} M) reduced the ^{3}H-outflow to approximately the same extent. Stimulation-induced outflow from artery rings preloaded with ^{3}H-NA in the presence of cocaine (10^{-5} M) or in the cold (3°C) approximated the Ca^{++}, bretylium and tetrodotoxin-insensitive [illegible]. These treatments all completely blocked the neurogenic contractile response. When artery rings were preloaded with ^{3}H-NA in the presence of normetanephrine (10^{-4} M), the stimulation-induced Ca^{++}-insensitive outflows were markedly reduced. The results suggest that the field stimulation-induced release of ^{3}H-NA is in part of extraneuronal origin.

Key words: Bretylium, calcium deprivation, cocaine, cold, electrical-field stimulation, extraneuronal release, neuronal release, ^{3}H-noradrenaline release, pulmonary artery, tetrodotoxin

The *in vitro* release of neurotransmitter from postganglionic sympathetic neurones elicited by nerve stimulation is commonly studied by labelling the preparation and presumably the adrenergic neurones with radioactive noradrenaline (^{3}H-NA). This method involves the following steps: (1) loading of the tissue with ^{3}H NA, (2) a wash-out period, in which supposedly non-specifically bound ^{3}H-NA is removed, (3) stimulation of the adrenergic neurones by electrical-field stimulation, (4) fractional collection of ^{3}H-outflow, and (5)

determination of total tritium or H NA and its H-metabolites by column chroma graphy and liquid scintillation counting. This elaborate method has been used for se blood vessels the rabbit main pulmonary artery (Su and Bevan 1970 Nedergaard r Schrold 1973 Starke *et al* 1974 1975), rabbit aorta (Bevan *et al* 1972, Schrold r Nedergaard 1976), rabbit ear artery (Allen *et al* 1973), dog arteries and veins (Vanho *et al* 1973 Vanhoutte 1974), human blood vessels (Stjärne and Gripe 1973, Stjärne e Brundin 1975)

The utility of this method rests on the assumption *inter alia* that the radioactive N bound selectively at the site of transmitter storage and released from it upon nerve stimul tion (Osswald 1976). The selectivity of the neuronal origin can be ascertained by mean *e.g* tetrodotoxin (TTX) an agent which selectively blocks axonal conduction in vasc smooth muscle preparations (Gershon 1967) or bretylium, an adrenergic neurone block agent It has been reported that electrical-field stimulation induced H-outflow is invariably abolished in the rabbit pulmonary artery by TTX and bretylium (Su and Bev 1970). Using the same artery we (Nedergaard and Schrold 1973) and others (Starke *et* 1974 1975) used stimulation that caused supramaximal excitation of adrenergic neuro with respect to tension development However neither of these authors specified whe the H-outflow represented selective transmitter release from adrenergic neurones. To o surprise we have later found that bretylium did not fully abolish the stimulation-induc H-outflow

There is another puzzling aspect of the stimulation induced H-outflow from rab pulmonary artery preloaded with ³H NA a marked decrease in the amount of relea radioactivity occurs with successive stimulations of the preparation, although the contract tension responses remain stable (Su and Bevan 1970, Nedergaard and Schrold 1973)

We therefore decided to examine in detail the neuronal selectivity of the ³H-outflow elicited by electrical-field stimulation of the rabbit pulmonary artery preloaded with ³H-NA Our results indicate that the H-outflow represents release from neuronal and extraneuronal sites.

Preliminary reports of this work were presented at the Sixth International Congress of Pharmacology Helsinki (Schrold and Nedergaard 1975 a) and at the Second International Symposium on Vascular Neuroeffector Mechanisms, Odense (Schrold and Nedergaard 1975 b).

Methods

Drugs The following drugs were used Bretylium tosylate (B rroughs Wellcome and Co., Tuckahoe U.S.A.) cocaine hydrochloride 3,4-dihydro yphenylglycol (DOPEG Sigma, Saint Louis, U.S.A.) (−)-noradrenaline hydrochloride (NA Fluka, Buchs, Switzerland) (−)-7 H noradrenaline (³H-NA 9.2 Ci/mmol The Radiochemical Centre, Amersham) (±)-normetanephrine hydrochloride (NMN Sigma, Saint Louis, U.S.A.) and tetrodotoxin (TTX Sigma, Saint Louis, U.S.A). The purity of H NA was checked by alumina column chromatography nd was found to be above 90 pure

Salt solution. The composition (mM) of the physiological salt sol tion (PSS) w N 144.. K 4.9 Ca^{++} 1.3, Mg^{++} 1.2, Cl^- 126.7 HCO 25 0 SO_4^{--} 1.2 glucose, 11 1 ascorbic acid 0.11 and calcium disodium ethylenediaminetetraacetic acid ($CaNa_2EDTA$), 0 03 Ca -free PSS Calcium (chloride) omitted and CaNa EDTA was replaced by Na_2EDTA (0 03 mM) The solutio aerated w bon dioxide (/v) l oxygen nd had pH 7 4.

…ration. A modification of the method described previously was used (Nedergaard and Schrold 1973). …main pulmonary artery was excised from unconscious exsanguinated rabbits. It was cleaned of excess …nd periarterial tissue and immersed in PSS. The artery was divided into 2 almost equal sized rings, …h are denoted in the following proximal and distal with reference to their position relative to the myo…ium. Each ring was suitably mounted on a plastic holder and connected by a platinum stirrup to …sducer. Each tissue was placed in a jacketed plastic bath filled with PSS (2.0 ml) maintained at 37 C. …bath could automatically be emptied and filled with prewarmed (37°C) PSS at a constant time …val.

…ulation. The rings were subjected to electrical-field stimulation using a Grass S48 square wave …ulator in connection with a constant current unit (S. Jacobsen, Odense University). This unit was …ered by the Grass stimulator and ensured that the current flow (250 mA) delivered was constant …ependent of the variable impedance existing in the tissue bath. In preliminary experiments, it was ob…ed that the impedance varied from bath to bath and for the same bath from preparation to prepara…. This variability is in part probably related to the geometric arrangement of the tissue relative to the …ulating electrodes. Two platinum electrodes were placed on either side of the arterial ring. Each prepa…ion was stimulated four to six times (S_1 to S_6) in a standard manner (trains of 300 monophasic pulses …se duration, 0.3 ms, frequency 10 Hz; current, 250 mA) with a rest period of 13.5 min between each …ulation period. Pulses were monitored on an oscilloscope (Hewlett Packard 1201 A). The stimulation …meters provided supramaximal stimulation of adrenergic nervous fibres as regards the contractile …ponse (Nedergaard and Schrold 1973).

…ording. The isometric contractions of the vascular smooth muscle were recorded by means of isometric …ce displacement transducer (Statham, G10B±0.15–350) connected to a linear pen recorder (Sargent …lch, model SRG). The rings were stretched at 3 g tension for 10 min and subsequently maintained at 1 g …ting tension. The preparations were allowed to rest for at least 20 min before proceeding. At the end of …ch experiment, NA (2.5×10^{-4} M) was added to the PSS in order to elicit the maximal obtainable …ntraction response.

…-outflow. The arterial rings were incubated with ^{3}H NA (10^{-4} M) for 45 min. Then the bath was auto…atically emptied and refilled with PSS (1.75 ml) every 2 min for the remainder of the experiment. The …gs were stimulated for the first time (S_1) after a wash-out period of 100 min (*vide supra*). The bath fluid …s collected directly in counting vials by means of a fraction collector (J. Cappelen, Odense University). At the end of the collection period the rings were removed from the bath and cut open into rectangles …hich were blotted under pressure (50 g) for 10 sec on filter paper previously moistened with PSS, trans…rred to a 25 ml polyethylene liquid scintillation counting vial and the wet weight (range 12.9–79.5 mg) …as determined. After addition of 0.50 ml Protosol (Nuclear Chicago Corporation) the closed vials stood …r 16 to 20 h at room temperature to allow for partial dissolution of the tissue. To each tissue-containing …al was added 18 ml of a fluor solution with the following composition: Liquifluor (Packard), 42 ml, …solute methanol, reagent grade, 100 ml and toluene, reagent grade to 1 liter. To the fraction-containing …als were added 16 ml of a scintillation fluid composed as follows: 2,5-diphenyloxazole (PPO, Hopkin & …illiams, Chadwell Heath, England), 5.0 g; 1,4-di-(2-(4-methyl-5-phenyloxazolyl))-benzene (Dimethyl-POPOP, Koch Light Laboratories, Colnbrook, England), 0.1 g, Triton X 100, technical grade, 333 ml and …ylene, technical grade, to 1 liter.

Tritium was measured with a liquid scintillation spectrometer (Mark II, Nuclear Chicago Corporation). Quenching was determined by adding known amounts of ^3H_2O or ^{3}H-toluene to representative samples. Stimulation-induced peak ^{3}H-outflow was calculated by summation of each fraction value which entered in the formation of the peak minus estimated passive outflow. The latter was calculated by using the mean …lue of the two fractions obtained just prior to each stimulation. The outflow of tritium was corrected for tissue weight, counting efficiency and specific activity and expressed as pmol g^{-1} tissue or as per cent of total tritium content in the tissue at the onset of each stimulation. The latter was calculated by summation of assayed tritium outflow and tritium content at the end of an experiment.

Determination of ^{3}H-NA and ^{3}H-metabolites

In the expts. involving determination of ^{3}H-NA and its ^{3}H-metabolites only 2 periods of stimulation (S_1 and S_2) were applied. The content of ^{3}H-NA, ^{3}H-DOPEG, ^{3}H-OMDA and total tritium was measured in the two fractions just before each stimulation (passive efflux: P′ and P″) and in the fraction during stimulation (S).

determination of total tritium or H NA and its H-metabolites by column chromatography and liquid scintillation counting. This elaborate method has been used for several blood vessels the rabbit main pulmonary artery (Su and Bevan 1970 Nedergaard and Schrold 1973 Starke *et al* 1974 1975), rabbit aorta (Bevan *et al* 1972, Schrold and Nedergaard 1976), rabbit ear artery (Allen *et al* 1973), dog arteries and veins (Vanhoutte *et al* 1973 Vanhoutte 1974) human blood vessels (Stjärne and Gripe 1973 Stjärne and Brundin 1975)

The utility of this method rests on the assumption *inter alia* that the radioactive NA is bound selectively at the site of transmitter storage and released from it upon nerve stimulation (Oswald 1976) The selectivity of the neuronal origin can be ascertained by means of *e.g.* tetrodotoxin (TTX), an agent which selectively blocks axonal conduction in various smooth muscle preparations (Gershon 1967) or bretylium, an adrenergic neurone blocking agent It has been reported that electrical-field stimulation induced H-outflow was invariably abolished in the rabbit pulmonary artery by TTX and bretylium (Su and Bevan 1970) Using the same artery we (Nedergaard and Schrold 1973) and others (Starke *et al* 1974 1975) used stimulation that caused supramaximal excitation of adrenergic neurones with respect to tension development. However neither of these authors specified whether the H-outflow represented selective transmitter release from adrenergic neurones. To our surprise, we have later found that bretylium did not fully abolish the stimulation-induced H-outflow

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Salt solution The composition (mM) of the physiol gical salt sol tion (PSS) was. N 144.2 K 4.9 Ca^{++} 1 3 Mg^{++} 1.2 Cl^- 126 7 HCO 25 0 SO_4^{2-} 1.., glucose, 11 1 ascorbic acid 0.11 and calcium disodium ethylenediaminetetraacetic acid (C Na_2EDTA), 0.03 Ca free PSS Calci m (chloride) was omitted and $CaNa_2$EDTA was replaced by Na_2EDTA (0 03 mM) The solutions were aerated with 5 carbon di xide (/v) in oxygen and had pH 7 4

Preparation. A modification of the method described previously was used (Nedergaard and Schrold 1977). The main pulmonary artery was excised from unconscious exsanguinated rabbits. It was cleaned of excess fat and periarterial tissue and immersed in PSS. The artery was divided into 2 almost equal sized rings, which are denoted as the following proximal and distal with reference to their position relative to the myocardium. Each ring was suitably mounted on a plastic holder and connected by a platinum stirrup to a transducer. Each tissue was placed in a jacketed plastic bath filled with PSS (2.0 ml) maintained at 37 C. The bath could automatically be emptied and filled with prewarmed (37°C) PSS at a constant time interval.

Stimulation. The rings were subjected to electrical-field stimulation using a Grass S48 square wave stimulator in connection with a constant current unit (S. Jacobsen, Odense University). This unit was triggered by the Grass stimulator and ensured that the current flow (250 mA) delivered was constant independent of the variable impedance existing in the tissue bath. In preliminary experiments, it was observed that the impedance varied from bath to bath and for the same bath from preparation to preparation. This variability is in part probably related to the geometric arrangement of the tissue relative to the stimulating electrodes. Two platinum electrodes were placed on either side of the arterial ring. Each preparation was stimulated four to six times (S_1 to S_6) in a standard manner (train of 300 monophasic pulses, pulse duration, 0.3 ms, frequency 10 Hz; current, 250 mA) with a rest period of 13.5 min between each stimulation period. Pulses were monitored on an oscilloscope (Hewlett Packard 1201 A). The stimulation parameters provided supramaximal stimulation of adrenergic neurone fibres as regards the contractile response (Nedergaard and Schrold 1977).

Recording. The isometric contractions of the vascular smooth muscle were recorded by means of a force displacement transducer (Statham, G10B±0.15–350) connected to a linear pen recorder (Sargent Welch, model SRG). The rings were stretched at 3 g tension for 10 min and subsequently maintained at 1 g resting tension. The preparations were allowed to rest for at least 20 min before proceeding. At the end of each experiment, NA ($2.5 \cdot 10^{-4}$ M) was added to the PSS in order to elicit the maximal obtainable contraction response.

³H-outflow. The arterial rings were incubated with ³H-NA (10^{-7} M) for 45 min. Then the bath was automatically emptied and refilled with PSS (1.75 ml) every 2 min for the remainder of the experiment. The rings were stimulated for the first time (S_1) after a wash-out period of 100 min (vide supra). The bath fluid was collected directly in counting vials by means of a fraction collector (J. Cappelen, Odense University).

At the end of the collection period the rings were removed from the bath and cut open into rectangles. These were blotted under pressure (50 g) for 10 sec on filter paper previously moistened with PSS, transferred to 25 ml polyethylene liquid scintillation counting vial and the wet weight (range: 12.9–29.5 mg) was determined. After addition of 0.50 ml Protosol (Nuclear Chicago Corporation) the closed vials stood for 16 to 20 h at room temperature to allow for partial dissolution of the tissue. To each tissue-containing vial was added 18 ml of a fluor solution with the following composition: Liquifluor (Packard), 42 ml, absolute methanol, reagent grade, 300 ml, and toluene, reagent grade to 1 liter. To the fraction-containing vials were added 16 ml of a scintillation fluid composed as follows: 2,5-diphenyloxazole (PPO: Hopkin & Williams, Chadwell Heath, England), 5.0 g; 1,4-di-(2-(4-methyl-5-phenyloxazolyl))-benzene (Dimethyl-POPOP: Koch Light Laboratories, Colnbrook, England), 0.1 g, Triton X 100, technical grade, 333 ml and xylene, technical grade, to 1 liter.

Tritium was measured with a liquid scintillation spectrometer (Mark II, Nuclear Chicago Corporation). Quenching was determined by adding known amounts of ³H₂O or ³H-toluene to representative samples. Stimulation-induced peak ³H-outflow was calculated by summation of each fraction value which entered in the formation of the peak minus estimated passive outflow. The latter was calculated by using the mean value of the two fractions obtained just prior to each stimulation. The outflow of tritium was corrected for tissue weight, counting efficiency and specific activity and expressed as pmol g⁻¹ tissue or as per cent of total tritium content in the tissue at the onset of each stimulation. The latter was calculated by summation of assayed tritium outflow and tritium content at the end of an experiment.

Determination of ³H-NA and its ³H-metabolites

In the experiments involving determination of ³H-NA and its ³H-metabolites only 2 periods of stimulation (S_1 and S_2) were applied. The content of ³H-NA, ³H-DOPEG, ³H-OMDA and total tritium was measured in the two fractions just before each stimulation (passive efflux: P' and P'') and in the fraction during stimulation (S).

Each of the two-minute fractions (P P' and S) was collected in cooled (0°C) tubes. Total tritium determined by removing an aliquot (100 μl). To each cooled fraction was added 20 μg each of NA, DOPEG and NMN as carriers. The fractions were acidified with acetic acid (1 N) to pH 3–4 and frozen (H overnight before being submitted to chromatographic analysis.

^{3}H NA and its ^{3}H metabolites were separated by the method of Graefe *et al.* (1973), which was slightly modified. Al_2O_3 was activated according to the method of Weil-Malherbe (1971). Each alumina column (diameter 5 mm) was prepared beforehand by washing 200 mg of activated Al_2O_3 with 5 ml sodium acetate (0.. M), pH 8.2. The fractions were thawed and after addition of 0.1 ml Na_2EDTA (10 .. M) they were adjusted to pH 8.2 with NH OH (2.5% and 0.25 .. v/v) during continuous stirring. Immediately thereafter each fraction was poured onto an alumina column, which was washed successively with 1 ml sodium acetate (0.2 M) and 2 ml H_2O The effluent and subsequent washings from the column contain ^{3}H O-methylated (NMN) and ^{3}H O-methylated plus deaminated metabolites (VMA and MOPEG).

Each column was now placed over a Dowex column (50W×4 15 5 mm H⁺, pH 2.5), which had been washed beforehand with ml H O ^{3}H NA and H DOPEG were eluted from the alumina column by separate additions of 1 ml acetic acid (0.5 N). The eluate dripped directly onto the Dowex column. H DOPEG a neutral catechol, was then found in the effluent and first washing (2 ml H_2O) from the Dowex column. The latter was washed further with .. ml H_2O which was discarded. H NA was then eluted from the column by repeated additions (4 times) of 1 ml HCl (2 N). H DOMA was not determined.

The H-content of various collected effluents and eluates (^{3}H-OMDA ^{3}H NA ^{3}H DOPEG) was determined by removing 2 ml aliquots and subjecting them to liquid scintillation spectrometry.

The recovery of NA, DOPEG and OMDA, the latter estimated by determination of NMN, was constant. The mean recovery ±S.E. was as follows. NA 80.7±0.7% (=22) DOPEG 85 3 1 (n=14) and NMN 97 6±0.9% (=14). All data were corrected for these recoveries.

The outflow of H NA and its ^{3}H-metabolites evoked by field stimulation was calculated as a percentage of induced total tritium according to the following formula.

$$\frac{S - \frac{1}{2}(P' + P'')}{S_T - \frac{1}{2}(P_T + P_T)} \times 100$$

where P and P' represent radioactivity in the two passive efflux fractions just prior to stimulation, S the H-content in the fraction during stimulation. Subscripts X and T represent radioactivity of H NA or ^{3}H-metabolites and total tritium, respectively All data were converted to dpm.

Statistical analysis. Student's *t*-test (paired or unpaired samples) or analysis of variance was used to compare differences between means.

Results

Effect of electrical-field stimulation on H-outflow and concomitant contractile response

Controls The stimulation induced H-outflow expressed either as pmol g⁻ (Fig. 1) or as of tissue H-content (Table I), declined markedly from the first to the second period of electrical-field stimulation (P<0 01) The H-outflows evoked by stimulation periods 2-4 remained approximately constant. In contrast, the concomitant tension response expressed either in gram (Fig. 1) or as of maximal obtainable response to NA (Table I) increased initially (P<0.01 S_1 versus S P<0.05 S versus S_3). Thereafter the tension response remained constant.

There was no difference (P>0.05) between each of the stimulation-induced H-outflow (S_1 to S_4) from proximal and distal rings, except that the H-outflow as % was larger from proximal rings than that from distal rings during S (Fig. 1 Table I). The tension response in gram obtained with proximal rings was always significantly higher (P<0.01 S_1 to S_4

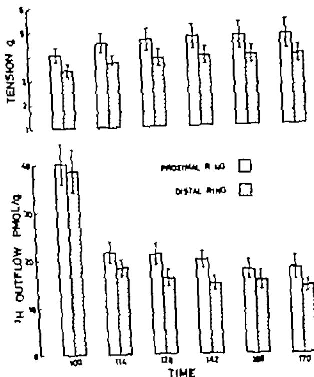


Fig. 1. Effect of electrical-field stimulations on tension response and concomitant ^{3}H-outflow from rabbit isolated pulmonary artery preloaded with ^{3}H-NA. *Ordinate* (upper graph): Mean contractile tension (g); (lower graph): Mean stimulation-induced ^{3}H-outflow (pmol g^{-1}). *Abscissa*: Time (min) after commencement of wash-out period. Open columns, proximal rings; cross-hatched columns, distal rings. The resting ^{3}H-outflows (pmol g^{-1} 2 min^{-1}) before S_1 and S_6 are (mean ± S.E.): 16.7 ± 1.9 and 10.8 ± 1.1 (proximal rings) and 16.3 ± 1.6 and 10.3 ± 1.0 (distal rings). The tissue ^{3}H-contents before S_1 and S_6 are given in Table I. Vertical bars denote S.E. Each column is the mean of 6 determinations.

($P < 0.05$, S_1 to S_6) than that obtained with distal rings (Fig. 1). However, the response of proximal and distal rings was found to be equal when the tension development was expressed as % of maximum (Table I).

Ca^{2+}-deprivation. Omission of Ca^{2+} from the PSS caused a complete inhibition of the contractile response to electrical-field stimulation (S_1 to S_6). However, ^{3}H-outflows were still induced (Fig. 2, Table II). The initial outflow (S_1) was 4 to 8 times higher than the subsequent outflows (S_2 to S_6, $P < 0.01$ S_1 vs S_2 to S_6). The latter decreased slightly with time. In two instances (S_3 and S_4) the ^{3}H-outflows from distal rings were larger than that from proximal rings (Table II). In the four others there was no difference ($P > 0.05$).

In 4 expts. (data from proximal and distal rings pooled) the rings were stimulated an additional time (S_7) using 1 000 pulses instead of 300. Raising the number of pulses increased the ^{3}H-outflow to 8.5 ± 1.3 pmol g^{-1} or 0.43 ± 0.08 % of the tritium content of the artery rings, *i.e.* significantly higher ($P < 0.05$) than S_6 (see Table II).

When the stimulation-induced ^{3}H-outflow from control preparations was subtracted the

TABLE I Electrical-field stimulation induced H-outflow and concomitant contractile response of isolated pulmonary arteries. Artery rings were incubated with H NA (10^{-} M) for 45 After a 100 min wash-out period the rings were stimulated 6 times (S_1 to S_6).

Ring		Proximal		Distal	
Tissue ³H-content					
S_1		2 100 ± 252 pmol g⁻		1 951 ± 231 pmol g⁻¹	
S_6		1 495 ± 194 pmol g⁻¹		1 390 ± 180 pmol g⁻¹	
Passive ³H-outflow[b]					
S_1		0.80 ± 0.03 %		0.85 ± 0.03 %	
S_6		0.74 ± 0.04 %		0.77 ± 0.05 %	
Treatment	Time[c]	H-outflow[d] %	Δ Tension %	H-outflow %	Δ Tension
Field stimulation					
S_1	100	1 93 ± 0 06	37 0 ± 3.3	1 98 ± 0 04	32 7 ± 2 5
S	114	1.20 ± 0 05	42.3 ± 4.2	1.03 ± 0.04	37.2 ± 3 2
S_3	128	1 18 ± 0 10	44.2 ± 4 9	0.97 ± 0.10	39 7 ± 3 7
S_4	142	1.21 ± 0.10	45 0 ± 5.4	0.95 ± 0 05	39 8 ± 3 9
S_5	156	1 13 ± 0.09	44.5 ± 6 0	1.02 ± 0 03	40 5 ± 4 1
S_6	170	1.20 ± 0.08	44.3 ± 6 3	0.97 ± 0.09	40 3 ± 4 1
NA[f]	184	10 8 ± 0 79	100	10.9 ± 0 64	100

Tissue H-content just before (100 min) S and (170 min) S_6

[b] Passive ³H-outflow expressed as % of tissue H-content just before (96–100 min) S_1 nd (166–170 min

Time fter onset of wash-out

[d] Stimulation-induced H-outflow expressed as % of tissue H-content at the start of the correspo stimulation period

Stimulation-induced tension response expressed as % of that obtained with a maximally effective centration (2.5 10^{-4} M) of NA

[f] H-outflow elicited by ex genous NA (2.5 10^{-4} M)

Values represent mean ± S.E. (n = 6).

mean H-outflow value found during the equivalent period of stimulation in Ca -free (column 1 minus column 2 in Fig. 2), the stimulation induced ³H-outflow sensitive to C deprivation was determined to be (mean ± S E.) 13 1 ± 3 5 (S) 12.2 ± 1 5 (S_3) 12.5 ± (S_4) 13.3 ± 1 7 (S) 12.7 ± 1 6 (S_5) and 13 0 ± 1 6 (S). There was no difference (P > 0 between these H-outflows (S to S_6)

Effect of bretylium and tetrodotoxin

Bretylium (3×10^{-4} M) and TTX (10^{-4} M) reduced the H-outflow evoked by stimulat (S_1 to S_6) to approximately the same extent as Ca -deprivation did (Fig. 2) The ti H-content was smaller in both the bretylium and TTX-treated preparations compa with that of the rings in Ca -free PSS (Table III). Thus, the H-outflow expressed as tissue H-content was higher than that in Ca -free PSS Furthermore, the H-outfl expressed as % of tissue content during S_1 to S_6 was rather constant and decreased less t in Ca -free medium (Table III)

Bretylium and TTX caused a total inhibition of the contractile response to stimulati while the response to exogenous NA was equal to that obtained with control preparatio This confirmed that the field stimulation did not excite the vascular smooth muscle its

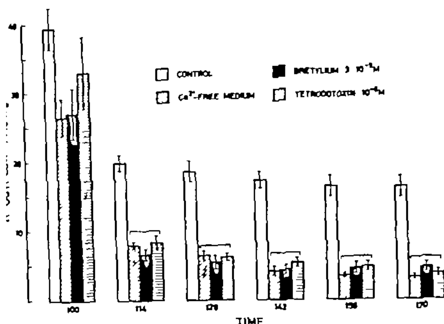


Fig. 2 Effect of calcium deprivation, bretylium or tetrodotoxin on electrical-field stimulation induced ³H outflow from rabbit isolated pulmonary artery preloaded with ³H-NA. Ordinate: Mean stimulation-induced ³H-outflow (pmol g^{-1}). Abscissa: Time (min) after commencement of wash-out period. Bretylium (10^{-5} M) and tetrodotoxin (10^{-6} M) were added 100 and 40 min, respectively, prior to S_1 (100 min). Ca^{2+}-free medium: The normal PSS was switched to Ca^{2+}-free PSS at the beginning of the passive efflux period (0 min). All treatments were maintained during the remainder of the expt. The passive ³H-outflow (pmol g^{-1} 2 min^{-1}) before S_1 and S_6 were (mean ± S.E.): 16.5 ± 1.2 and 10.6 ± 0.7 (control); 25.5 ± 1.5 and 19 ± 0.6 (Ca^{2+}-free), 17.9 ± 1.6 and 10.8 ± 1.2 (bretylium) and 22.9 ± 0.8 and 13.3 ± 0.5 (TTX). The tissue ³H-contents before S_1 are given in Table III. Each column is the mean of 3–12 determinations (control, 12 (the results from proximal and distal rings in Fig. 1 were pooled); Ca^{2+}-free PSS, 10; bretylium, tetrodotoxin, 3). Vertical bars denote ± S.E. The level of significance for the differences between test and control data is indicated. No symbol, Not significant, $P > 0.05$; $P < 0.05$; $P < 0.01$; $P < 0.001$.

Effect of cold, cocaine and cocaine plus normetanephrine

Electrical-field stimulation of artery rings preloaded with ³H NA in the cold (3 C) or in the presence of cocaine (10^{-4} M) caused ³H-outflow (pmol g^{-1}) (Fig. 3), which was of the same magnitude as that found in Ca^{2+}-free PSS (Fig. 2). When NMN (10^{-4} M) was present besides cocaine (10^{-4} M) the outflows were further reduced, but only significantly in the periods related to S_1 and S.

The ³H-outflows expressed as % of tissue ³H-content (not shown) were larger following these treatments than those obtained in Ca^{2+}-free PSS. However the tissue ³H-content was only about 25% of that found with preparations in Ca^{2+}-free PSS (see legend Fig. 3).

Effect of normetanephrine

When NMN (10^{-4} M) was present during the preloading of the arteries with ³H-NA it markedly reduced the stimulation-induced ³H-outflows in Ca^{2+}-free PSS. This is apparent

TABLE II Effect of calcium deprivation on electrical-field stimulation induced ³H-outflow from ... isolated pulmonary arteries. Artery rings were incubated with ³H NA (10^{-4} M) for 45 min normal PSS. Then, the PSS was replaced by Ca⁺⁺-free PSS containing Na_2EDTA (3 M) which was used throughout the remainder of the expt. The treatment schedule was described in Table I

Ring		Proximal		Distal	
Tissue ³H-content[a]					
S_1		630±346 pmol g⁻¹		2 529±212 pmol g⁻¹	
S_6		1 909±297 pmol g⁻¹		1 890±181 pmol g⁻¹	
		pmol g⁻¹ 2 min⁻¹	%	pmol g⁻¹ 2 min⁻¹	
Passive ³H-outflow[b]					
S_1		27.1±..5	1.07±0.08	23.9±1.7	0.95±0
S_6		14.6±1.0	0.86±0.15	13.3±0.7	0.75±0
Treatment	Time[c]	³H-outflow[d]			
		pmol g⁻¹	%	pmol g⁻¹	
Field stimulation					
S_1	100	21.7±3.7	0.87±0.14	31.2±2.4	1.21±0
S_2	114	7.30±0.84	0.33±0.07	8.63±0.73	0.39±0
S_3	128	5.73±1.08	0.27±0.06	7.29±0.65	0.35
S_4	142	2.77±0.55	0.16±0.06	5.62±0.58	0.25±0
S_5	156	3.31±0.35	0.19±0.05	3.71±0.60	0.20±0
S_6	170	2.74±0.48	0.15±0.0.	3.78±0.36	0.22±0
NA	184	234±30	12.4±0.58	187±40	11.0±1

[a] Tissue ³H-content just before (100 min) S_1 and (170 min) S_6.
[b] Passive ³H-outflow expressed either as pmol g⁻¹ 2 min⁻¹ or as % of tissue ³H-content just before (96–100 min) S_1 and (166–170 min) S_6.
[c] Time after onset of wash-out.
[d] Stimulation-induced ³H-outflow expressed either as pmol g⁻¹ or as % of tissue ³H-content at the start of the corresponding stimulation period
[e] ³H-outflow elicited by exogenous NA (2.5×10^{-4} M).
Values represent mean±S.E. (n=5).

from Fig. 4 as regards the absolute amounts released, but it also applied to the outflow expressed as a percentage of the tissue ³H-content although the ³H-content just before was lower in experiments with NMN than in those without (Table III)

³H NA and its ³H-metabolites

In a separate series of expts. ³H NA and some of its ³H-metabolites were determined. ³H NA, ³H DOPEG and ³H-OMDA constituted each approximately 15 %, 15 % and 40 %, respectively of the total tritium in the passive efflux (Table IV) There was no difference between the distribution of the total tritium on ³H NA and its ³H-metabolites in the passive efflux prior to S_1 and S_6, respectively (Table IV) In contrast during electrical field stimulation at 3 Hz for 100 s the distribution of the net ³H-outflow on ³H NA and its ³H metabolites elicited by S_1 was quite different from that evoked by S_6. Thus, 11 % of the S_1 induced ³H-outflow was collected as ³H NA compared to 38 % during S_6, whereas ³H-OMDA

...LE III The effect of calcium deprivation, bretylium, tetrodotoxin or normetanephrine on electrical-field stimulation induced ^{3}H-outflow from rabbit pulmonary arteries. Artery rings were incubated with ^{3}H-NA (10^{-7} M) for 45 min in either normal PSS or PSS which contained normetanephrine (10^{-4} M). The latter was added 20 min before ^{3}H-NA. Then the tissues were washed with either normal or Ca^{2+}-free PSS. Bretylium and TTX were added to the PSS during the wash-out period. Treatments were continued for the remainder of the expt. After 100 min wash-out period the tissues were stimulated 4 or 6 times (S_1 to S_6).

...tment	n	Tissue ^{3}H content[a] pmol g^{-1}	Passive[b] ^{3}H outflow	^{3}H-outflow (%) induced by field stimulation[c,d] S_1	S_2	S_3	S_4	S_5	S_6
...e[e]	[illegible]	2826±165	0.82±0.02	1.93±0.04	1.11±0.04	1.08±0.08	1.08±0.07	1.07±0.05	1.12±0.06
...-free ...S	10	NS 2579±192	1.01±0.05	1.07±0.13	0.36±0.05	0.31±0.04	0.23±0.04	0.20±0.03	0.18±0.02
...ium[g]	4	NS 2593±269	NS 1.18±0.12	NS 1.75±0.18	0.49±0.08	0.46±0.12	0.39±0.09	0.41±0.03	0.38±0.04
...odo-...in	3	NS 1706±115	NS 1.36±0.13	NS 1.93±0.32	0.56±0.10	0.46±0.07	0.43±0.08	0.42±0.09	0.37±0.08
...-free ...S ...meta-...phrine[i,j]	6	1312±96	NS 1.31±0.15	0.48±0.06	0.14±0.02	0.09±0.02	0.12±0.02		

...issue ^{3}H-content just before (100 min) S_1.
...ssive ^{3}H-outflow expressed as % of tissue ^{3}H-content just before (96–100 min) S_1.
...mulation-induced ^{3}H-outflow expressed as % of tissue ^{3}H-content at the start of the corresponding ...ulation period.
...he artery rings were stimulated 6 times (S_1 to S_6) at 100, 114, 128, 142, 156 and 170 min after the onset ...wash-out (cf. Tables I and II).
...formal PSS. The results from proximal and distal rings in Table I were pooled.
...Ca^{2+} was omitted from the PSS. The results from proximal and distal rings in Table II were pooled.
...retylium ($3 \cdot 10^{-6}$ M) was added 100 min before S_1.
...etrodotoxin (10^{-6} M) was added 40 min before S_1.
...Ca^{2+}-free PSS and normetanephrine (10^{-4} M).
...he data in this horizontal column were compared with those obtained in Ca^{2+}-free PSS.
...ues represent mean ± S.E. * $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

...resented 74% of S_1-induced ^{3}H-outflow compared to 38% during S_1 (Table IV $S_{...}$ ...ures). The stimulation-induced ^{3}H-outflows were 51 800±2 800 dpm (S_1) and 10 800± ...00 dpm (S_4) ($P < 0.001$, $N = 6$). Accordingly the absolute amount of ^{3}H NA released by ...and S_4 was the same: 5 630±670 dpm (S_1) and 4 100±500 dpm (S_4) ($P > 0.05$, $N = 6$).

Discussion

...e present work provides strong evidence for the view that the ^{3}H-outflow induced by ...ectrical-field stimulation of isolated rabbit main pulmonary artery preloaded with ^{3}H NA ...of neuronal as well as extraneuronal origin.

That NA is released from nerve terminals in rabbit pulmonary artery is already well ...tablished (Bevan *et al.* 1969). This vessel is innervated only by postganglionic adrenergic ...d constrictor sympathetic fibres (Bevan and Su 1964, Verity and Bevan 1968).

That amount by which the stimulation-induced ^{3}H-outflow is reduced by bretylium (by ...locking NA-release from adrenergic neurones; Boura and Green 1959, Hertting *et al.*

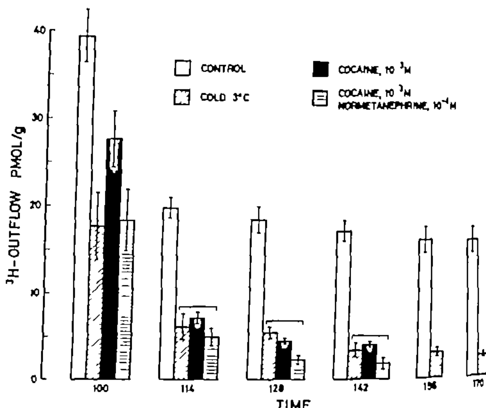
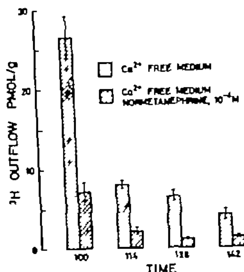


Fig. 3 Effect of cold, cocaine or normetanephrine on electrical-field stimulation induced ^{3}H-outflow from isolated rabbit pulmonary artery preloaded with ^{3}H NA. Ordinate: Mean stimulation-induced ^{3}H-outflow (pmol g^{-1}). Abscissa: Time (min) after commencement of wash-out period. Cocaine (10^{-3} M), cocaine (10^{-3} M) plus normetanephrine (10^{-4} M) and cold (3°C) was applied 20 min prior to the addition of ^{3}H NA (10^{-6} M). All treatments were maintained during the remainder of the expt. The pre-stimulation ^{3}H-outflows (pmol g^{-1} 2 min^{-1}) and tissue ^{3}H-contents (pmol g^{-1}) before S_1 were (mean ± S.E.): 16.5 ± ... and 2 026 ± 165 (control), 11.9 ± 1.6 and 484 ± 46 (cold), 15.2 ± 1.9 and 590 ± 67 (cocaine) and 16.2 ± ... and 522 ± 96 (cocaine + normetanephrine). Each column is the mean of 4–12 determinations (control) 12, cold 4, cocaine 4, cocaine plus normetanephrine 4). Vertical bars denote ± S.E. No mark: Not significant $P > 0.05$ $P < 0.01$ $P < 0.001$

1962), TTX (by blocking axonal action potentials, Narahashi 1974) or omission of Ca from the bath fluid (Ca is a prerequisite for stimulation-evoked NA-release, Kirpekar 1974) is considered proportional to the release of ^{3}H NA from adrenergic neurones.

The following findings suggest that the ^{3}H-outflow induced by electrical-field stimulation of the pulmonary artery preloaded with ^{3}H NA in part represents transmitter release from extraneuronal binding sites. *First* TTX failed to abolish the stimulation-induced outflow (Table III Fig. 2) in a concentration that blocked the response to transmural stimulation. TTX likewise failed to abolish the stimulation-induced ^{3}H-outflow from the perfused rabbit ear artery preloaded with ^{3}H NA (Allen *et al* 1973). In contrast, TTX in a lower concentration than we used (10^{-6} M) abolished the ^{3}H NA outflow as well as contractions induced by transmural stimulation of the rabbit pulmonary artery (Su and Bevan 1970). This was also the case for human blood vessels (Stjärne and Gripe 1973, Stjärne and Brundin 1975). *Secondly* bretylium failed to abolish the stimulation induced outflow (Fig. 2, Table III) while it blocked the contractile tension responses. On the other hand, bretylium consistently



4 Effect of normetanephrine on the Ca^{2+}-insensitive ³H-outflow induced by electrical-field stimulation olated rabbit pulmonary artery. Ordinate: Mean stimulation-induced ³H-outflow (pmol g^{-1}). Abscissa, e (min) after the commencement of the wash-out period. Ca^{2+}-free PSS was used. Normetanephrine added 20 min prior to the incubation with ³H-NA (10^{-6} M). The passive ³H-outflows (pmol g^{-1} min^{-1}) before S_1 were (mean ± S.E.): 25.5 ± 1.5 (Ca^{2+}-free) and 20.4 ± 3.6 (Ca^{2+}-free + normetanephrine). tissue ³H-contents before S_1 are given in Table III. Each column is the mean of 6–10 determinations ntrol 10, normetanephrine 6). Vertical bars denote ± S.E. P 0.01 P 0.001.

BLE IV Metabolism of ³H-NA released spontaneously and by electrical-field stimulation of rabbit iso-lated pulmonary arteries. Artery rings were incubated with ³H-NA (10^{-6} M) for 45 min. After 100 min wash-out period the rings were stimulated at 100 min (S_1) and at 114 min (S_2).

eatment	Percent of total radioactivity											
	NA				DOPEG				OMDA			
	P′	P″	S	S_{corr}	P′	P″	S	S_{corr}	P′	P″	S	S_{corr}
ld stimulation												
S_1	12.8 ±2.1	14.0 ±2.3	11.4 ±1.3	11.0 ±1.3	13.6 ±1.0	14.9 ±0.8	6.3 ±0.6	3.5 ±0.5	41.4 ±3.4	37.3 ±2.9	64.6 ±2.5	73.8 ±3.7
S_2	15.2 ±2.7	16.5 ±2.8	25.1 ±2.2	31.2 ±2.8	18.0 ±1.6	17.6 ±1.6	13.4 ±1.8	7.7 ±2.5	42.3 ±1.6	39.3 ±1.6	44.1 ±5.6	38.9 ±8.9

IA ³H-noradrenaline. DOPEG ³H-3,4-dihydroxyphenylglycol. OMDA: ³H-O-methylated and de-aminated metabolites.
′ and P″ represent radioactivity in the next to the last and the last fraction, respectively just prior to stimulation.
S represents the stimulation-induced release of radioactivity in the fraction during stimulation.
S_{corr} represents the stimulation-induced release of radioactivity during S corrected for passive efflux (for calculation see Methods).
Values represent mean ± S.E. (6). The level of significance for the difference between S_1 and S_2 is as indicated. No symbol: Not significant; P > 0.05 P 0.01; P 0.001

abolished both the H NA outflow and the contractile response to transmural stim (Su and Bevan 1970) *Thirdly* Ca^{++}-*deprivation failed to abolish the stimulation-i* outflow (Fig. 2, Table II) The discrepancies between our results and those of Su and (1970) with regard to the effect of TTX and bretylium on H-outflow cannot rea explained. Su and Bevan (1970) used a *superfusion technique* while we empl fractional collection-method (Nedergaard and Schrold 1973) It is unlikely that t crepancies are due to this difference in experimental conditions. Using the superfusio nique we have recently found that bretylium did not abolish the H-outflow (Schr Nedergaard, unpublished). The different results may possibly be related to the stim used and the time at which the effect of adrenergic neurone blocking agents were ined Su and Bevan (1970) apparently stimulated their preparation several times with train of pulses (1 200 *cs* ours 300) before the adrenergic neuron blocking agent wa ed at the end of the expt. (*cf* their Fig. 4). This stimulation procedure may prefer have released tritium from extraneuronal sites. This possibility gains support fro recent finding that when the pulmonary artery ring was first stimulated intensely th tylium markedly reduced the stimulation induced H-outflow (Schrold and Nedergaa published) The failure of bretylium and TTX to abolish stimulation-induced H-outfl not be due to submaximal treatment This is supported by the findings that these trea caused a block of the neurogenic tension response. Furthermore, the H-outflow g⁻) was approximately the same for both these treatments and Ca^{++}-deprivation (*indicating that the same maximal effect on H-outflow was achieved*

Fourthly the H-outflows could be evoked by stimulation of artery rings which ha preloaded with H NA in the cold or in the presence of cocaine (Fig. 3). The upt adrenergic neurones via the membrane pump is inhibited by cold or by cocaine (Nede and Bevan 1971) Thus, it may be inferred that the H NA was taken up and bo extraneuronal sites from which it was released by field stimulation.

NA is taken up and bound by neuronal (uptake-1) as well as by extraneuronal site as vascular smooth muscle (uptake 2) and connective tissue (Avakian *et al* 1968, Nede and Bevan 1971 Powis 1973). When the artery rings were preloaded with H NA presence of a specific uptake 2 inhibitor NMN (Iversen 1965) the stimulation-i H-outflows in Ca -free PSS were markedly reduced (Fig. 4) This suggests that the of H NA originates in part from uptake 2 binding sites. Direct evidence for the vie the stimulation induced H-outflow from a blood vessel preloaded with H NA orig in part *from extraneuronal sites has recently been obtained (Schrold* and Neder 1976) When rabbit aortic tunica media, which contains no adrenergic neurones, wa loaded with H NA, field stimulation caused a Ca insensitive H-outflow

The H-outflows and tension responses induced by electrical-field stimulation sho clear dissociation (Table I Fig. 1). The stimulation induced H-outflows declined mar after the first period of stimulation and then remained almost constant In contras concomitant tension responses increased initially and then remained approximatel same. Su and Bevan (1967 1970) *using the superfused rabbit pulmonary artery* likewise found that the H-outflow evoked by the initial period of field stimulatior disproportionately great compared to those elicited by subsequent periods of stimula

le the tension responses remained constant. On the other hand, Starke et al. (1974) using the same preparation found that the H-outflow induced by S_1 was 90% of that ...ced by S_2.

A disproportionately great S_1-induced ³H-outflow was also found in Ca⁺⁺-free medium (Fig. 2), in the presence of bretylium (Fig. 2) or TTX (Fig. 2), or under conditions (cold and ...caine; Fig. 3) where uptake-1 was inhibited during the preloading period with ³H NA. When the extraneuronal ³H-outflows (Ca⁺⁺-insensitive) were subtracted from the corresponding outflows from untreated tissue, the calculated H-outflows were the same in ... six periods (cf Fig. 2, column 1 minus column 2). These ³H-outflows most likely represent release of ³H-NA from adrenergic neurones. This concept also provides closer correlation between neuronal H-outflows and concomitant tension responses. Additional direct evidence for this view was obtained by the finding that the absolute amount of H NA released by S_1 and S_2 was the same. The small increase in the initial tension responses cannot be related to a corresponding increase in ³H NA release, but must be due to other factors.

Since the initial stimulation-induced H-outflow (S_1) represents a disproportionately high amount of tritium from extraneuronal sites compared to subsequent outflows, it is particularly unsuited for studies of release of H NA from adrenergic neurones. In the light of this, results from studies (Schrold and Nedergaard 1973, 1974) in which S_1 has been used need to be reexamined.

The view that the initial stimulation-induced ³H-outflow (S_1) is predominantly of extraneuronal origin gains further support from the finding that the distribution of the total H-outflow on ³H NA and its H-metabolites elicited by S_1 was quite different from that of S_2 (Table IV). This is in contrast to Su and Bevan (1970), who found an almost equal percentage proportion of H-NA released during S_1 and S_2. Since these authors likewise found a much greater total H-outflow during S_1 than S_2, the absolute amount of H-NA released by S_1 must have been greater than that of S_2. This seems surprising, since Su and Bevan found that S_1 and S_2 elicited a tension response of equal size.

H-NA and ³H-DOPEG represented approximately 15% each of the total H-efflux (Table IV). Su and Bevan (1970) using the superfused rabbit pulmonary artery strip found that ³H-NA constituted 30 % of the total tritium in the superfusate. However this value is in good accordance with ours, since their determination of H-NA included ³H-DOPEG. A direct comparison of the values found during stimulation seems less relevant, because different stimulation parameters were used.

The mechanism of release of ³H NA and its metabolites from extraneuronal sites can only be guessed at. It could be due to a local depolarization causing sufficient release of K which in turn triggers the ³H-outflow. Alternatively it may be likened to the electrophoretic release from an ion exchange resin.

³H-outflow of extraneuronal origin may possibly also occur in other tissues. Thus, Katz and Kopin (1969) found that field stimulation induced H-outflow from rat atria preloaded with ³H NA also had a Ca⁺⁺-insensitive component.

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Experimental Cardiac Hypertrophy and the Synthesis of Poly(A)-Containing RNA and of Myocardial Proteins in the Rat: The Effect of Digitoxin Treatment

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Abstract

TURTO, H *Experimental cardiac hypertrophy and the synthesis of poly(A) containing R[NA] and of myocardial proteins in the rat The effect of digitoxin treatment* Acta phy[siol] scand 1977 *101* 144–154

The synthesis of poly(A) co taining RNA was increased in heart of non-digitalized and digitalized [rats] after ortic constriction, the increase being of the same degree as that of the RNA lacking this seq[uence]. No differences were found, either i the absence or presence of polyuridylic acid, in the incorporatio[n of] radioactivity into protein by cardiac ribosomes isolated from animals treated differently. It wa[s] concluded, that after the constriction of the aorta the synthesis of mRNA proceeds at a similar rate a[s that] of the bulk RNA, and that the treatment of the animals with digitoxin does not bolish the stimul[us of] hypertrophy

As the heart adapts to work-overload caused by constriction of the aorta, ribonucleic [acid] (Koide *et al* 1969 Fanburg *et al* 1968) and protein synthesis (Posner *et al* 1968, Fla[...] *et al* 1971) are stimulated in the myocardium. A positive correlation between the incr[ease] in the heart mass and both the increase in RNA concentration (Nair *et al* 1969) and [the] increase in myocardial protein synthesis (Zak *et al* 1971) has been observed. However [the] treatment of the animal with digitoxin has been shown to prevent the increase in heart m[ass] (Williams *et al* 1965 Turto *et al* 1973) but without marked effect on RNA synth[esis] (Posner *et al* 1968 Turto *et al* 1973).

After the onset of work-load, the synthesis of ribosomal RNA (rRNA) and tran[sfer] RNA (tRNA) increases rapidly but the distribution of the radioactive label in various ty[pes] of RNA (28S, 18S and 4S) has been shown to be the same for the hearts of animals with [and] without aortic constriction (Fanburg *et al* 1968 Koide *et al* 1969) Moroz (1967) and Z[ak] *et al* (1971) did not find any evidence for more active ribosomal fractions or for larger p[ro]portions of ribosomal aggregates as a result of hypertrophy However Meerson *et* [*al*] (1974) were able to observe an increased ratio of translating po[...] to nontranslati[ng]

somal monomers and subunits and the authors suggested that the synthesis of mRNA increase to a greater extent than the synthesis of rRNA.

the present study myocardial RNA synthesis in non-digitalized and digitalized rats aortic constriction has been studied. Cytoplasmic and nuclear RNA were isolated by thermal phenol method (Markov *et al.* 1973). Polyadenylic acid (poly(A)) containing A from these two fractions was isolated using oligodeoxythymidylate (oligo(DT))-lose columns. Also the incorporation of radioactivity from aminoacyl-tRNA into pro-s by ribosomes isolated from the hearts of non-digitalized and digitalized rats with and out constriction of aorta was measured.

Material and Methods

le albino rats of the Wistar strain, two month of age and weighing about 200 g, were used. Half of the nals received digitoxin (0.1 mg/100 g b. wt.), dissolved in mixture of ethanol glycerol water (1 : 1 : 1), for 6 days each week. The other animals received the same amount of solvent alone. Digitalization was ted four days before operation.

onstriction of the aorta. Half of the non-digitalized and digitalized animals underwent constriction of abdominal aorta as described earlier (Turto *et al.* 1973). The other half of the animals underwent m operation.

abelling of RNA. At indicated times after the operation the animals were injected i.p. with μCi/g b wt. carrier free [^{32}P]H_3PO_4 (Amersham Radiochemical Center) diluted into 1 ml of saline. The animals were apitated 4 h later and the hearts were removed. The atria and great vessels were cut away and the rts were rinsed rapidly in saline, blotted dry and weighed.

Determination of total RNA. Total RNA was determined by the orcinol method (Albaum *et al.* 1945) as cribed earlier (Turto *et al.* 1973).

Extraction and purification of RNA from heart. For sucrose density gradient analyses, RNA was extracted the method of Posner and Fanburg (1966). The tissue was minced with scissors in ice-cold 20 mM s-HCl buffer (pH 7.2) and homogenized in 20 volumes of the buffer with teflon-glass Potter-Elvehjem mogenizer. The homogenate was made 0.5 with respect to sodiumdodecylsulphate (SDS) and was racted with an equal volume of 88 phenol containing 0.1 8-hydroxyquinoline for 30 min at 4°C. The ueous phase was re-extracted with phenol, and after adding one tenth volume of 20 sodium acetate (H 5.2) RNA was precipitated with 2.5 volumes of 95 ethanol at −20°C for 4 h.

DNA as removed from the precipitate by treatment with 20 μg of RNAase free DNAase (Sigma emical Co) for 30 min at room temperature in 2 ml of 20 mM Tris-HCl (pH 7.2) containing 20 mM SO_4. After adding ice-cold water and SDS to final concentration of 0.5 the mixture was depro-nized with phenol, and RNA was precipitated from the aqueous phase with sodium acetate and ethanol. graded DNA was removed from the precipitate by 2 precipitations with ethanol (final concentration) at high salt concentration (Posner *et al.* 1966) and the final precipitate was dissolved in 2 sodium etate (pH 5.2).

For the preparation of poly(A) containing RNA, cytoplasmic and nuclear RNA were extracted by the thermal phenol method (Markov *et al.* 1973; Georgiev 1967). Heart tissue, 5 to 7 g, was homogenized in volumes of ice-cold 0.14 M NaCl and an equal volume of water-saturated phenol (pH 6.2) containing 1 8-hydroxyquinoline was added. After shaking for 15 min at 4°C the phases were separated by centri-gation. The aqueous phase was deproteinized and the interphase layer was re-extracted at 4°C. The queous phase was discarded and the interphase was extracted with 0.14 M NaCl and phenol at 75°C. The queous phases were deproteinized two times in the presence of 1 SDS, once with phenol chloroform (1 : 1, v/v), once with chloroform and finally the RNA was precipitated with 3 volumes of ethanol con-taining 2 sodium acetate at −20°C overnight. DNAase treatment was carried out as described before.

Sucrose density gradient analysis of RNA. About 200 μg of RNA in 0.2 ml of 2 sodium acetate was layered on 5 ml of 5–20 linear sucrose gradient, made in 2 sodium acetate (pH 5.2) (Munro *et al.* 1964). The tubes were centrifuged in Spinco SW 50 rotor at 24 000 rpm for 14 h. Fractions (25–27 in number) were collected by puncturing the bottom of the tube. Optical density was measured at 260 nm

with the Zeiss M4 QIII spectrophotometer after adding distilled water Absorbancy of blank fractio subtracted from each reading. Direction of sedimentation in figures is from right to left.

RNA was precipitated with 10% trichloroacetic acid (TCA) after adding 100 μg of carrier yeas The precipitate was collected on Whatman glass-fiber f lter washed with 50 ml of cold 5% TCA a of 80% ethanol The f lter was dried dissolved in 0.5 ml of Soluene 100 (Packard) and counted i Gel (Packard)

Ol go(dT) f actionation of RNA RNA containing poly(A) sequences is known to bind to ol by base pairing (Edmonds *et al* 1969). No more than 30 A_{260} units of RNA were dissolved in 0 01 HCl buffer (pH 7.5) containing 0.4 M NaCl and was applied to a 1.0 ml oligo(dT)-cellulose (Collaborative Research) previously washed with the application buffer The non-absorbed fracti collected and the column was washed with the application buffer The bound fraction was elu 0 01 M Tris HCl buffer (Aviv *et al* 1972) and the absorbancy at 260 nm was measured. After the of carrier RNA the fractions were precipitated with 10 % TCA, collected on glass f ber filters and p for radioactive counting as described above.

Preparation of ATP from heart tissue Tissue ATP was prepared essentially as described by Faub (1968). Potato apyrase (Sigma), 0.5 mg/ml, was used to hydrolyze tissue ATP and the resulting Al separated by Dowex 1-formate chromatography (Bergkvist *et al* 1954). The purity of AMP was by paper electrophoresis (Klouwen 1962) AMP prepared in this manner represents the AMP por tissue ATP since the amount of free AMP in tissue is negligible (Threlf ll 1957).

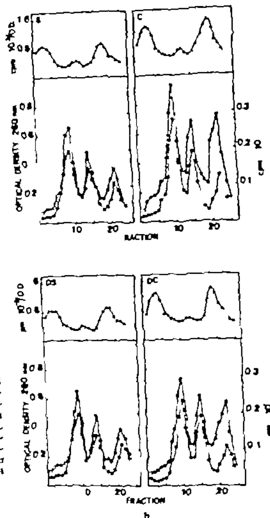
The specific activity of precursor ATP (determined as AMP) was used to correct RNA la Percent stimulation in RNA labelling was calculated by subtracting the RNA AMP ratio of sham-o animals from that of the animals with constriction of the aorta and dividing the result by the RNA ratio of sham-operated a imals and multiplying by 100. The units for RNA and AMP are cpm

Preparation of heart muscle ribosomes. For the preparation of ribosomes (Castles *et al.* 1972) th tissue was finely minced with a razor blade and homogenized in a buffer contai g 50 mM Tr (pH 7 6), 12.5 mM $MgCl_2$, 250 mM KCl and 250 mM sucrose with a teflon-glass Potter-El homogenizer After centrif gation t 13 000 *g* for 10 min the pellet was re-extracted with the homo tion medium and the combined supernatants were centrif ged at 78 000 *g* for 2 h. The resulting pel suspended in medium A havi g the same composition as that of the homogenization medium exce the concentration of KCl was reduced to 80 mM The suspension was treated with Triton X 100 and deo ycholate (final concentrations 0.5%, and 1%, respectively). The precipitate of myosin as remo centrifugation for 15 min at 13 000 *g* and 7 ml of the supernatant was layered over 5 ml of 1 M suc medium A The ribosomes were pelleted by centrifugation at 105 000 *g* for 4 h.

For sucrose density gradient analysis, ribosomes were loaded on the top of a 15 to 30 % linear gradient prepared in medium A, and the tubes were centrifuged for 90 min at 37 500 rpm in a Spi 50 rotor Twenty-five fractions were collected and the optical density at 260 nm was determi

Preparation of labelled aminoacyl-tRNA Aminoacyl-tRNA was prepared using tRNA extracte rat liver (Deutscher 1974) nd the enzyme fraction prepared from rat l ver (Wigle 1973). The tRN charged with L[4 5 H]leucine [^{14}C]phenylalanine nd 19 additional amino acids as descri Wigle (1973). The specific radioactivity of labelled aminoacyl-tRNA was calculated from its absorp 260 nm (μg of RNA/ml = OD_{260}/0 0221) and its radioactivity The supernatant protei (transfer preparation) was prepared from the 78 000 *g* supernatant of rat liver homogenate (Castles *et al.* 1972

Protei synthetic acti ity of ribosome in vitro The ratio of A_{260}/A_{280} of the ribosomal prepa varied from 1 79 to 1.87 ndicating the purity of the ribosomes. RNA concentration in ribosom pensions was determined by mod fication (Wool *et al* 1967) of the method of Fleck *et al.* (1962). Th somes were d luted with medium A to the concentration required The assay of protei synthetic of ribosomes was carried out i a tot l volume of 1 0 ml contai ing 50 μmol of Tris-HCl (pH 7.8), 8 of KCl, 12.5 μmol of $MgCl_2$, 10 μmol of 2-mercaptoethanol, 1 μmol of ATP 0 4 μmol of GTP 1 of creatine phosphate, 0 1 mg of creatine phosphokinase, 100 μg of tRNA labelled w th [^{3}H]leuc [^{14}C]phenylalanine and 19 additional amino acids, d 2.5 mg of s pernatant protein. The amo polyuridylic acid, when present was 100 μg. The reaction was initiated by adding 0 1 ml of ribo suspension. The incubation was for 30 min t 37°C water bath shaker When y of these cond were changed, the details of the changes made re given in the legends to the f gures. The reactio terminated by adding 0.1 ml of 3 M KOH and the ncubation was continued for 30 min. The samples were prepared for co nting (Castles *et al* 1972), dissolved in Soluene 100 and counted i Insta



1. Pattern of labelling of myocardial . two days after the operation in non-lized rats with (C) and without (S) con-ion of aorta, and digitalized rats with) and without (DS) construction of aorta. l circles counts/min, solid circles = optical density, solid triangles = specific radio-ity (obtained by dividing the counts at . fraction by the optical density of that tion)

Results

nstriction of the aorta resulted in an increase in the ratio of heart weight to body weight, : increase being about 10% at 2 days and about 19% at 4 days after constriction of the rta in non-digitalized animals. In digitalized animals the increase was about 4% at 2 days d 8% at 4 days, respectively

Also, an increase in the myocardial RNA concentration was observed 2 days after the nstriction of the aorta, the values (mg/g wet weight of tissue, mean ± S.D.) being .49 ± 0.18 in sham-operated, 2.82 ± 0.21 in animals with constriction of the aorta ($p < 0.02$), .34 0.17 in digitalized sham-operated and 2.58 ± 0.18 in digitalized animals with constric-ion of the aorta (p 0.05). At 4 days the values were 2.42 ± 0.16, 3.03 ± 0.31 ($p < 0.005$), .38 0.20 and 2.84 ± 0.24 ($p < 0.01$), respectively

TABLE I Labelling of RNA. Non-digitalized and digitalized (D) animals underwent either sham-op (S) or constriction of aorta (C). The specific activity of RNA was obtained by dividing the counts/min by the sum of optical densities of the entire sucrose gradient (Fig 1)

Group	Hypertrophy %	RNA cpm/OD	AMP cpm/OD	% increase in sp. activity of RNA
S		431	2 200	
C	9 6	719	2 820	30.1
DS		444	2 210	
DC	3.5	649	2 700	19 6

Fig. 1 demonstrates the labelling pattern of cardiac RNA two days after the ope RNA is separated into three peaks on a 5 to 20% sucrose density gradient, which ha shown to correspond in sedimentation characteristics (28S 18S and 4S) to the species from liver (Posner *et al* 1966) Any marked changes could not be seen labelling pattern of RNA extracted from the hearts of non-digitalized and dig animals with and without constriction of aorta. However after correcting them specific activity of AMP the extent of labelling of total RNA after aortic constricti 30% greater in non-digitalized animals and 20% greater in digitalized animal compared to the values obtained for the respective controls (Table I).

Phenol extraction and oligo(dT)-cellulose fractionation of RNA Two types of RN obtained by the thermal phenol method The RNA extracted at 4 C contained the 28 and 4S peaks, typical for the cytoplasmic RNA whereas the radioactivity of th extracted at 75 C was rather evenly distributed throughout the sucrose gradient considered to be of nuclear origin (Fig. 2) (Georgiev 1967 Markov *et al.* 1973). corporation of radioactivity into the oligo(dT) unbound and bound fractions of th plasmic and nuclear RNA was linear for at least 6 h. Also the increase in the labe AMP was linear for at least six hours following the injection of the label (Fig. specific radioactivity of the nuclear RNA was about two times the value for the cyto RNA and the specific radioactivity of RNA bound to oligo(dT) was about twice th for the unbound fraction (Table II)

The labelling of cytoplasmic and nuclear RNA containing poly(A) (the fraction t bound to oligo(dT)-cellulose) was increased by about 60% at 2 days and about 4 days after constriction of the aorta. In digitalized animals the increase was 44% an

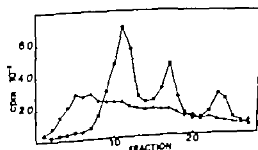
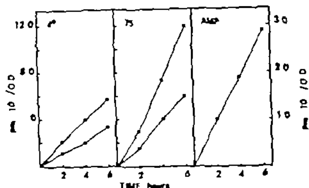


Fig. — The pattern of labelling of myocardi extracted t 4 C (■) and t 75°C (▲). The were injected with 1 mCi of [³²P]H PO an 4 h later



3 Time course of labelling of portion of tissue ATP and toplasmic (extracted at 4°C) nuclear (extracted at 75°C) A fractionated by the oligo-cellulose column (□) fraction RNA that not bound to (dT). (■) poly-(A) containing A

pectively The increase in the specific activity of RNA that was not bound to oligo(dT) of the same degree in both thermal fractions (Table II).

Protein synthesis by cardiac ribosomes in vitro. Sucrose density gradient analysis of diac ribosomes of non-digitalized and digitalized animals with aortic constriction did t show any substantial shift to larger polysomes if compared with the respective controls g 4). The assay of ribosomal activity *in vitro* showed that [³H]leucine was incorporated o protein rapidly for the first 10 min and thereafter the rate declined until after 30 min further synthesis occurred (Fig. 5 on the left).

The incorporation of radioactivity from [H]leucyl-tRNA into protein was directly oportional to the concentration of ribosomes t least up to 60 μg of ribosomal RNA/ml g 5 in the middle). The saturating amount of aminoacyl-tRNA for 30 μg of ribosomal NA was about 200 μg (Fig. 5, on the right). In the experiments, the amount of aminoacyl-NA (100 μg) was not saturating, but since the protein synthesis was linearly proportional the *concentration of ribosomes, it was not considered limiting.*

ABLE II. The effect of constriction of aorta and digitoxin treatment on the labelling of RNA fractionated by the oligo(dT) cellulose column The groups are marked as in Table I. The numbers in parentheses show he per cent increase in the specific radioactivity of RNA over the respective control

roup	Hyper-trophy	AMP cpm/OD	RNA extracted at 4°C		RNA extracted at 75°C	
			Not bound to oligo(dT) cpm/OD	Bound to oligo(dT) cpm/OD	Not bound to oligo(dT) cpm/OD	Bound to oligo(dT) cpm/OD
ve days after operation						
		210	293	590	412	1 160
	12	2 930	593 (53 1)	1 153 (58 4)	833 (52.5)	2 572 (66.5)
DS		2 065	235	496	381	1 232
DC	5	3 100	540 (40 5)	1 055 (41 7)	796 (39.5)	2 700 (46 1)
ee days after operation						
3		2 520	206	394	413	743
C	20	3 570	371 (26.7)	721 (28.9)	720 (22.8)	1 316 (26 8)
DS		2 260	216	376	386	658
DC	8	3 300	347 (18 0)	664 (20 6)	637 (13 3)	1 098 (13.7)

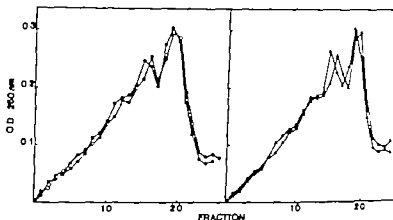


Fig. 4. Sucrose density gradient distribution of ribosomes isolated from the hearts of non-digitalized rats without (○) and with (●) constriction of the aorta, and of digitalized rats without (Δ) and with (▲) constriction of the aorta.

The ribosomes isolated from the hearts of different groups of animals had the same time course of incorporation of radioactivity into protein, they had the same optimum concentration for aminoacyl-tRNA and the incorporation of radioactivity into protein was directly proportional to the concentration of ribosomes. The addition of polyuridylic acid into the reaction mixture increased the incorporation of radioactivity from

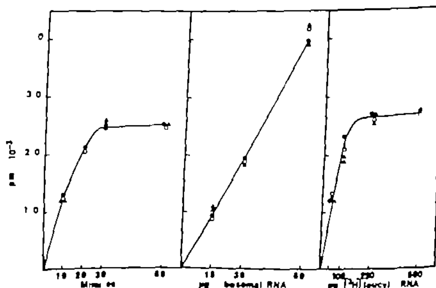


Fig. 5. Time course of incorporation of radioactivity from aminoacyl-tRNA into protein by cardiac ribosomes. The ribosomes, containing 30 μg of ribosomal RNA, were incubated with 100 μg [³H]leucyl-tRNA (59 635 cpm) for different time periods (on the left). The effect of concentration of ribosomes on the incorporation of radioactivity into protein. Different concentrations of ribosomes were incubated with 100 μg of [³H]leucyl-tRNA (59 635 cpm) for 30 min (in the middle). The effect of concentration of aminoacyl-tRNA on the incorporation of radioactivity into protein by cardiac ribosomes. Ribosomes containing 30 μg of ribosomal RNA, were incubated with different concentrations of [³H]leucyl-tRNA for 30 min (on the right). Ribosomes were isolated from the heart of non-digitalized rats without (○) and with (●) constriction of the aorta, and of digitalized rats without (Δ) and with

III. Incorporation of radioactivity from aminoacyl-tRNA into protein by ribosomes isolated four days after the operation from the heart tissue of non-digitalized and digitalized animals ith and without constriction of the aorta. Ribosomes, containing 30 μg of RNA were incubated with 100 μg of [^{3}H]leucyl-tRNA (59 635 cpm) or [^{14}C]phenylalanyl-tRNA (14 346 cpm) as described in Material and Methods. The concentration of poly(U), hen present, was 100 μg ml.

os of somes	Incubation time, min	Hypertrophy %	Incorporation, cpm		
			[^{3}H] leucyl-tRNA	[^{14}C] phenylalanyl-tRNA	
				− poly(U)	+ poly(U)
	10			857 ± 47	2 900 ± 202
	30		2 163 ± 173	1 404 ± 40	5 055 ± 144
	10			924 ± 63	2 822 ± 173
	30	18	2 230 ± 229	1 426 ± 61	5 208 ± 249
	10			880 ± 72	2 856 ± 145
	30		1 974 ± 189	1 375 ± 60	5 152 ± 252
	10			900 ± 50	2 886 ± 226
	30	7	2 135 ± 326	1 417 ± 69	5 101 ± 173

Mean ± S.D. of 4 determinations. Mean ± S.D. of 3 parallel determinations.

enylalanyl-tRNA into protein about threefold. However no substantial difference was und in the incorporation of radioactivity into protein by ribosomes isolated from different oups of animals (Table III).

Discussion

everal studies have demonstrated an early increase in the synthesis of myocardial RNA llowing the constriction of aorta and leading later to an increased RNA content of the art (Meerson 1969 Fanburg *et al.* 1968, Koide *et al.* 1969 Turto *et al* 1973). In the resent study a marked increase in the labelling of RNA was observed 2 days after the peration. The specific activity of α-phosphate of tissue ATP was used to correct the labelling f RNA. The adequacy with which this type of correction reflects precursor specific activity epends upon an assumption of the absence of compartmentalization of the precursor in the ssue and similar rates for the incorporation of other nucleotides into RNA as that for the denosine nucleotide.

Subsequent to the constriction of the aorta a generalized stimulation in the labelling of ll species of RNA throughout the entire sucrose gradient was found. No selective increase n the labelling was found in any particular area of the gradient. The results presented here re consistent with those of Fanburg *et al.* (1968) and Koide *et al.* (1969).

The earlier studies (Fanburg *et al* 1968, Koide *et al* 1969) did not reveal any evidence for n increased synthesis of mRNA in heart tissue undergoing hypertrophy as measured by the base composition analysis. Meerson *et al.* (1974) have suggested an increased synthesis of mRNA as a possible explanation for the finding of an increased ratio of translating to non-translating ribosomes in the hypertrophic heart. I the present study an approach was made to study mRNA synthesis by isolating poly(A) containing RNA by the oligo(dT)-cellulose column.

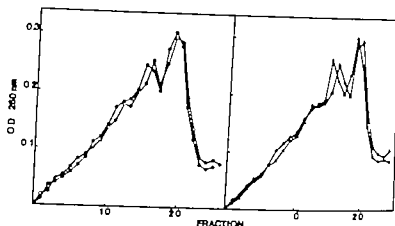


Fig. 4 Sucrose density gradient distribution of ribosomes isolated from the hearts of non-digital without (○) and with (●) constriction of the aorta, and of digitalized rats without (△) and with strictiction of the aorta.

The ribosomes isolated from the hearts of different groups of animals had the same course of incorporation of radioactivity into protein they had the same optimum centration for aminoacyl tRNA and the incorporation of radioactivity into prote directly proportional to the concentration of ribosomes. The addition of poly acid into the reaction mixture increased the incorporation of radioactivity from

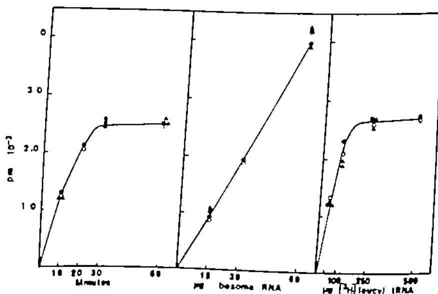


Fig. 5. Time course of incorporation of radioactivity from aminoacyl-tRNA into protein by ca ribosomes. The ribosomes, containing 30 μg of ribosomal RNA, were incubated with 100 μ [^{3}H]leucyl-tRNA (59 635 cpm) for different time periods (on the left). The effect of concentration of somes on the incorporation of radioactivity into protein. Different concentrations of ribosomes incubated with 100 μg of [^{3}H]leucyl-tRNA (59 635 cpm) for 30 min (in the middle). The effect of concentration of aminoacyl-tRNA on the incorporation of radioactivity into protein by cardiac r somes. Ribosomes containing 30 μg of ribosomal RNA were incubated with different concentration [^{3}H]leucyl-tRNA for 30 min (on the right). Ribosomes were isolated from the heart of non-digitalized without (○) and with (●) constriction of the aorta, and of digitalized rats without (△) and with constriction of aorta.

...ertrophy (Williams *et al.* 1965 Turto *et al.* 1973) and myocardial protein synthesis ...to *et al.* 1973) it has a smaller effect on RNA synthesis (Posner *et al.* 1968, Turto *et al.* ...) and on the increase in the activity of prolyl hydroxylase (Turto 1977). In the present ... treatment of the animals with digitoxin did not have effect on the incorporation of ...ctivity from aminoacyl-tRNA into proteins by cardiac ribosomes *in vitro*. The effect ...toxin treatment on the soluble factors in protein synthesis *in vitro* remains unsolved, ...e results of Moroz (1967) suggest a lack of dependence upon soluble factors, prepared ... different sources, in the changes of protein synthesis in cardiac hypertrophy

...estigation was supported by the National Research Council for Medical Sciences, Finland.

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A marked increase in the labelling of cytoplasmic and nuclear RNA was found i heart of non-digitalized and digitalized animals after the constriction of aorta The in in the specific radioactivity of RNA that was not bound to oligo(dT) was of the degree as that of the poly(A) containing RNA Due to the difficulties inherent in gentle homogenization of the heart tissue, some contamination of the cytoplasmic with nuclear RNA would be expected (Markov *et al* 1973) The variation in the amou poly(A) containing RNA (from 0.5 to 2% of the total RNA) may be due to its bindi proteins during extraction (Braverman *et al* 1972) or due to a contamination with dependent RNAase, capable to cleave the poly(A) segment (Rosenfeldt *et al* 1972). W RNA preparations are fractioned by a poly(A) selection procedure, the template ac for protein synthesis has been found to remain associated with the poly(A) contai molecules (Morrison *et al* 1972, Kazazian *et al* 1973) providing evidence for the ident tion of these molecules as mRNA. From the earlier studies (Fanburg *et al* 1969 Zak 1971) and the data presented here it may be concluded that there is an enhanced synthe mRNA as well as of rRNA and tRNA as a result of aortic constriction Evidenc increased synthesis of mRNA in the overloaded heart *in vitro* has been reported by Schr *et al* (1968) However the changes in RNA synthesis may reflect changes primarily in stitial cells, since autoradiographic studies show a predominant labelling of RNA in i stitial cells of the muscle tissue (Morkin 1971 Jablecki *et al* 1973).

Contradictory results have appeared regarding protein synthesis in the overloaded h Moroz (1967) has demonstrated that ribosomes isolated from hypertrophic hearts are m active in protein synthesis, as measured in an *in vitro* amino acid incorporating system. difference can entirely be explained by the higher content of ribosomes in microsome paration isolated from hypertrophic heart. No evidence was found of a larger proportio ribosomal aggregates Schreiber *et al* (1968) have found an increased incorporation radioactivity into proteins by ribosomes isolated from hearts subjected to pressure overl *in vitro* without evidence of larger ribosomal aggregates. Hjalmarsson *et al* (1972 a have reported an increase in protein synthesis in association with increased levels of p ribosomes in overloaded perfused hearts. Zak *et al* (1971) did not observe difference in relative amount of polyribosomes and in the ribosomal activity measured in an *in* amino acid incorporating system between the animals with aortic constriction and controls

The present results are consistent with those of Zak *et al* (1971) No evidence was fou of a larger proportion of ribosomal aggregates or of an increased incorporation of rad activity from aminoacyl-tRNA into protein by ribosomes isolated from hypertrophic he Zak *et al.* (1971) have also found an increase in the labelling and relative amount of membrane-bound ribosomes whereas the labelling of free ribosomes decreases during development of cardiac hypertrophy However it is unclear if the increase in the amou and the activity of the membrane-bound ribosomes is due to the increase in the rou endoplasmic reticulum in the muscle cells, or due to the increase in the population of int stitial cells, which can make up 75% of the total cell number but only a small percenta of the heart volume (Grove *et al* 1969)

... that although digitoxin treatment inhibits the d... of

...trophy (Williams *et al.* 1965 Turto *et al.* 1973) and myocardial protein synthesis *et al.* 1973) it has a smaller effect on RNA synthesis (Posner *et al.* 1968, Turto *et al.* and on the increase in the activity of prolyl hydroxylase (Turto 1977). In the present treatment of the animals with digitoxin did not have effect on the incorporation of ctivity from aminoacyl-tRNA into proteins by cardiac ribosomes *in vitro* The effect itoxin treatment on the soluble factors in protein synthesis *in vitro* remains unsolved, e results of Moroz (1967) suggest a lack of dependence upon soluble factors, prepared different sources, in the changes of protein synthesis in cardiac hypertrophy

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Relationship between Sarcomere Length and Active Force in Rabbit Papillary Muscle[1]

By

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Abstract

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tic peak twitch force (stimulation frequency 0.5/s; 29.5–30.5°C) as correlated with sarcomere in isolated papillary muscles of the rabbit. Sarcomere length was measured from photographic [illegible] (1.5 ms exposure time) performed at rest between contractions and at the time of isometric twitch force. The sarcomere length at rest as found to be relatively uniform throughout the prepara- [illegible] to be linearly related to the overall muscle length within the range L_{max} – 0.85 L_{max}. The distribu- of sarcomere lengths increased considerably as the muscle went from rest to activity. Studies of [illegible] markers showed different degrees of shortening (or elongation) of individual segments along the h of the preparation. The mean *resting* sarcomere length at L_{max} (the optimum muscle length for production) as 2.44 ± 0.01 µm (grand mean ± S.E., 7 muscles). The mean *active* sarcomere length [illegible] as 2.29 ± 0.04 µm. Active force declined steeply as the muscle length as reduced below L_{max} resting sarcomere length of 2.0 µm, active force was approximately 1/3 of the maximum. The [illegible]ved differences between the length–tension relationships in myocardium (twitch responses) and skel- muscle (tetanus contractions) are discussed on the basis of length dependency of the activation proc n cardiac muscle.

characteristic relationship between tetanic force and sarcomere length has previously n defined in single skeletal muscle fibres of the frog (Gordon, Huxley and Julian 1966, man 1966). Several attempts have been made to investigate whether similar relationship ts between peak twitch force and sarcomere length in mammalian myocardium. Studies this kind (Sonnenblick, Spiro, Cottrell 1963, Grimm and Whitehorn 1968 Grimm *et al.* 70) ere based on sarcomere length determinations on chemically fixed papillary muscles, d the results were therefore most likely complicated by length changes of the sarcomeres roduced by the histological procedure itself Furthermore, as the papillary muscle prepara n contains considerable series compliance (*e.g.* Sonnenblick 1967 Edman and Nilsson 68, 1969 Nilsson 1972, Julian and Sollins 1975 Krueger and Pollack 1975), which varies

[1] This study as performed during sabbatical leave of absence (June, 1974 June, 1975) given to A. F G. the Colleges of Dentistry and Medicine, University of Illinois at the Medical Center Chicago, Illinois, SA.

substantially with the techniques used for dissection and mounting, these earlier experi did not provide definite information as to the relationship between contractile force sarcomere length of the *active* myocardium

Winegrad (1974) and Pollack and Huntsman (1974) have demonstrated the pot value of microscopically examining living isolated cardiac muscle. A resting sarco length of 2.35 ± 0 02 (S E.) μm was found by Krueger and Pollack (1975) at optimum le for force production (L_{max}) in rat papillary muscles. Substantial sarcomere short occurred during activity along with stretching at the ends of their preparation where t damage was probable. Julian and Sollins (1975) studying the sarcomere length-te relationship in living papillary muscles from small rats, also found considerable int shortening during contraction. The active sarcomere length–tension relationship deriv the latter authors however was shifted to much shorter sarcomere lengths than that sented by Krueger and Pollack (1975) and exhibited a striking similarity with the le tension curve obtained for skeletal muscle.

The present investigation is an extension of our previous work (Grimm and Wohlfart I which established a resting sarcomere length at L_{max} of 2.38 ± 0.004 (S.E.) μm in the iso papillary muscle of the rat In the present study resting sarcomere lengths were deter along the ascending limb of the length–tension curve in isolated papillary muscles o rabbit. In addition active sarcomere lengths were determined at the peak of the iso twitch The results of this study suggest that active force of a papillary muscle is closely related to resting sarcomere length than to sarcomere length recorded during act

Methods

Papillary muscles were dissected from the right heart ventricle of young (0.5–0.8 kg) rabbits. The a were heparinized before their killing and exsanguination. The heart was remo ed within 2 minute the death of the animal and placed i an oxygenated bathing solution at about 35°C. The right ve was spl t pen and the dissection was carried out under a Zeiss stereo III microscope at 10 magnific A 6–7 mg loop of plati um wire (diameter 0.25 mm) was tied (braided silk thread, Ethicon 6 0) tendon f a selected pap llary muscle *in situ* close to the myotendinous junction. The muscle as then removed from the heart together with the piece of the ventricular wall to which t inserts. The musc held at slightly stretched length in the dissectio trough by means of thin needles placed throu tendon loop nd the ventricular wall block. The latter was trimmed to a size slightly wider than th of the muscle nd a U-shaped loop of platinum wire was tied to it Care was taken not to touch the n between ts insertions. The muscles used had nearly cylindrical shape over approximately 90 o length. The muscle widened t wards the base and tapered tow rds the tendinous end. Muscles th the appropriate shape nd were thin en ugh to provide a clear sarcomere pattern were found in abo out of three animals. The thickness of the muscles was 0.3–0 5 mm in the middle of the preparation overall length of the muscles at L_{max} (the length for maximum force production) was 2 0–5.3 mm

Muscle chamber mounting and temperature Th muscle was mounted horizont lly in a chamber be a force transducer and glass hook The chamber was continuously perfused (5 ml/min) ith a b solution that had been prewarmed to 30°C and was equilibriated with mixt re of 95 O_2 and 5 immediately before entering the trough. The bath temperature was 29.5–30.5°C in the different experi and did not ary by more tha ± 0.2°C throughout given experiment. The overall length of the m was adjusted by means of micrometer screw which controlled the position of the force transducer apparatus has been previ usly described (Edma and Jóhannsson 1976).

Tension recording Tension was recorded by means of tral gauge transducer (compliance 1 4 μm described previously (Edman and Jóha nsson 1976) The tension signal was recorded on a Grass poly ink writer The tension was also displayed on T ktronix 502 A oscilloscope and photographed on film.

...lution. The muscle was stimulated at frequency of 0.5/s by passing current between two platinum electrodes that were placed 1.7 mm apart on either side of the muscle. A unidirectional pulse (2 ms ...ion, 150% of threshold) was used.

...ution. The bathing solution had the following composition (mM): NaCl 100, KCl 5, $MgSO_4$ 1.5, ... 2.5 $NaHCO_3$ 25, NaH_2PO_4 1.5, Na acetate 20, glucose 10, insulin 2 U/l, pH 7.45. All chemicals of analytical grade and dissolved in deionized, double-distilled water.

...surements of sarcomere length. Light from 100 W microscope lamp (Zeiss) was passed through the ...ration. A monocular microscope tube provided with water immersion objective (Zeiss 40 X, N.A. ...) and Zeiss 20 X ocular lens were used for inspection of the sarcomere pattern. For photographic ...ding the microscope was fitted with an extension tube provided with side viewer and 35 mm Pentax ...ra. Kodak Tri-X film was used with an exposure time of 1.5 ms. The degree of magnification on the ...(90 X) was determined by photographing stage micrometer scale submerged in the bathing solution. ...regions of the preparation could be explored by moving the bath on the microscope stage. Clear sarco... patterns were obtained by focussing close to the upper surface of the muscle: the most distinct patterns ...found along the edges of the preparation. Pictures were taken in the fully relaxed state between contrac... and also during activity. In the latter case the exposure was timed to occur while the contractile force above 94 of peak isometric twitch amplitude. Measurements of the sarcomere length were made ... the film with Nikon model 6 C profile projector at 20 X magnification. A row of ten sarcomeres measured and mean value of the sarcomere length obtained. A minimum of 5 measurements were ... at each muscle length.

...surements of muscle segments and of overall muscle length. The overall muscle length was measured ...om the points of attachment of the platinum loops. Such measurement was made at 10 X magnifica... in the Nikon comparator from photographic record of the muscle adjusted to L_{max}. Changes in muscle ...th were determined from the setting of the micrometer screw which controlled the position of the force ...sducer.

...some experiments segmental length changes were studied as the muscle went from rest to full isometric ...traction. For this purpose markers of silk filaments (20 μm thick and 200–300 μm long) were placed on ... muscle surface. Photographic records (see above) of the muscle with its natural and artificial markers ... taken in the relaxed state between contractions and at the time of peak isometric force. Measurements ... the distances between the markers were carried out in the Nikon comparator.

Experimental procedure. After being mounted the muscle was stimulated to contract at frequency of ... for about 1 h before the actual experiment was started. L_{max} was determined by using the following ...cedure. The muscle was stretched in steps of 0.05 mm (approximately 2 of the muscle length) until ...ximum active force was recorded and further increment in length caused reduction of active force. ...tures of the sarcomere pattern were taken when the peak twitch tension had attained steady-state level. ... muscle length was then reduced to give approximately 85, 70, 55, and 40 of optimum peak twitch ...sion and the sarcomere pattern was photographed at each length. After each length change the muscle ...readjusted to its optimum length and new microphotographs were taken. The sarcomere length measured ... L_{max} did not differ significantly (at the 5 level) during the course of an experiment. At the end of ...w experiments sarcomere length recordings were also made after the muscle had been stretched beyond ... to produce approximately 90 of optimum active force.

Results

...arcomere length in resting muscle

...hotographic records of the sarcomere pattern from three different regions of a papillary ...muscle between contractions are illustrated in Fig. 1. Measurements from such pictures in a ...Nikon comparator at 20 X magnification showed that the resting sarcomere lengths at opti...mum length for force production, L_{max}, was relatively uniform in different regions of the ...preparation. It should be pointed out that some regions of the muscle, usually along the ...midline and near the base, did not exhibit clear sarcomere patterns due to the thickness of the ...preparation and thus could not be judged as to the uniformity of sarcomere length. However ...measurements from homogenized fixed rat papillary muscles (Grimm and Wohlfart 1974) demonstrated uniformity between the periphery and the core of the preparation.

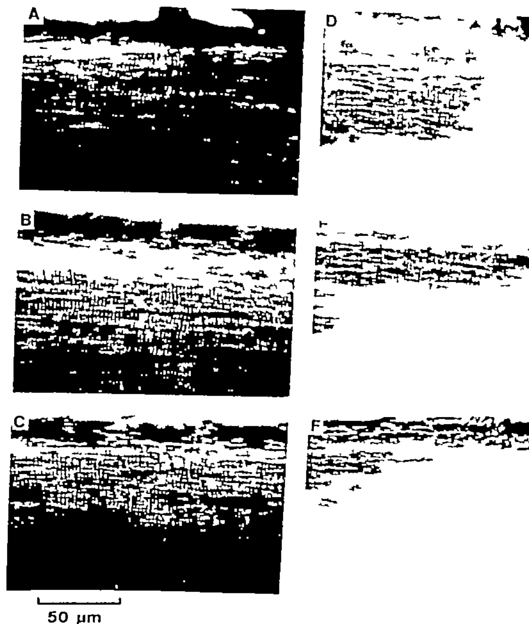


Fig. 1 Sarcomere patterns from three different regions of papillary muscle in the relaxed state bet contractions (left panel, A B, C) and t the peak of isometric contraction (right panel, D, E, F) pictures, 1.5 ms exposure time. A, D are close t the tendon while B, E and C, F are from segments m middle portion of the preparation.

In Table I the resting sarcomere lengths at L_{max} (mean ± S D) are presented for papil muscles from each of 7 animals. Measurements (usually 5) were made each time the mu length was reestablished at L_{max} after the muscle had been studied at a shorter length. Si the sarcomere length determinations were not statistically different between the determi tions at L_{max} and no muscle slippage could be demonstrated, these mean values per mus were pooled. As seen in Table I the mean resting sarcomere length at the beginning of t descending limb of the length tension curve (just before a decrease in force production

1. Sarcomere lengths (SL, μm) at optimum muscle length (L_{max}) at rest and peak activity. Number of measurements in each individual muscle is given within parentheses.

	Rest Mean $SL_R \pm$ S D	Activity Mean $SL_A \pm$ S D	Ratio SL_A/SL_R
	2 43 ± 0 06 (22)	2 34 ± 0 08 (24)	0 96
	2.31 ± 0 05 (20)	2.22 ± 0 09 (20)	0 88
	2.43 ± 0 06 (5)	2.28 ± 0 08 (5)	0 94
	2 45 ± 0 08 (20)	2 [illegible] ± 0 09 (20)	0.93
	2 40 ± 0 06 (30)	[illegible]10 ± 0 15 (30)	0.88
	[illegible]47 ± 0 03 (25)	2.38 ± 0 11 (25)	0 96
	2 41 ± 0 05 (25)	2 44 ± 0 10 (31)	1 01
[illegible] mean	2.44 ± 0 01 (S.E.)	2.29 ± 0.04 (S E.)	0.94

[illegible]ment, see Methods) is 2.44 ± 0.01 (S.E.). This value is the grand mean of the mean values [illegible] 7 reported muscles without any weighting for the number of individual measurements. [illegible]g. 2 illustrates the relation between overall muscle length and sarcomere length at rest [illegible]termined in 5 muscles. As indicated by the regression line drawn from the data ($n = 217$), [illegible]e were proportional changes in sarcomere length and overall muscle length. Thus, for a percental change in muscle length (within the range L_{max} – 0.85 L_{max}) almost the same change in sarcomere length was obtained. At approximately 85% L_{max} corruga-[illegible]s of the muscle surface and waviness of the myofibrils could be observed microscopically corrugations of the surface of the muscle were irregular the distance between the folds times the sarcomere length.

[illegible]ig. 3 shows the relationship between peak twitch tension and resting sarcomere length papillary muscles. As can be seen there is a relatively moderate scatter of the data points [illegible]ined from different experiments. There was a steep decline in tension as the sarcomere was reduced below 2.4 μm. A 50% decrease in peak twitch force was obtained by resting sarcomere length to 2.05–2.25 μm in the various experiments.

[illegible] length in acti[illegible] muscle

[illegible] sarcomere length during activity was measured from photographic records taken at the [illegible]e of isometric peak twitch force (see Methods). Whereas the sarcomere pattern at rest [illegible] quite uniform, there was considerable variation of the sarcomere length in different [illegible]ons of the muscle during contraction. Furthermore, as can be seen from Fig. 1 (D–F), register of the sarcomeres became less distinct at the height of activity The mean sarco-[illegible] length measured during activity at L_{max} is given for the different muscles in Table 1 [illegible]an be seen that the standard deviations of sarcomere lengths measured during activity are [illegible]siderably (mean 76 [illegible]) higher than corresponding data obtained from the muscles at rest. [illegible]s finding supports the view that there is a wider dispersion of sarcomere length during [illegible]vity The mean active sarcomere length at L_{max} determined from all 7 muscles was 2.29 ± [illegible]4 μm (S.E.).

[illegible]he differential behaviour of the sarcomeres along the length of the muscle was further [illegible]died by recording the distances between surface markers on the muscle. The behaviour

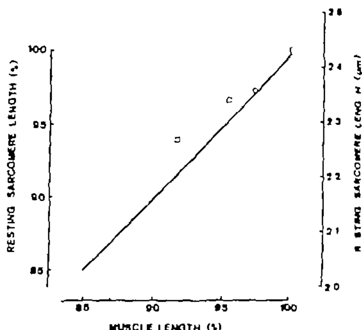


Fig. 2. Relation between resting sarcomere length (S.L.) and overall muscle length (M.L.) of 5 papillary muscles. L_{max} is taken as 100 percent. The different symbols represent different muscles and each point is the mean value of 5–30 measurements. The straight line is the regression of S.L. on M.L. determined from the pooled data (n = 217). Its equation is S.L. (μm) = 0.0263 M.L. ( ) − 0.21. The standard deviation about the line is 0.07 μm.

of individual segments recorded in two different muscles is shown in Fig. 4. It can be seen that whereas some regions shortened, other regions actually elongated or remained constant as the muscle went from rest to full activity. An examination of different muscles failed to reveal a consistent and uniform pattern of shortening between muscles. It is noteworthy that the behaviour of the end segments was not different from that of other regions of the preparation. No consistent elongation of the ends of the muscles was observed (cf. Krueger and Pollack 1975). This is probably due to the fact that the metal loops used for mounting of the muscle did not come into direct contact with the muscle tissue (see Methods) to cause any damage to the contractile material of the end regions of the preparation.

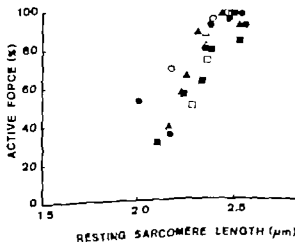
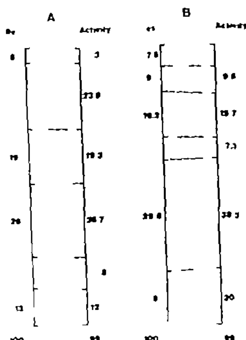
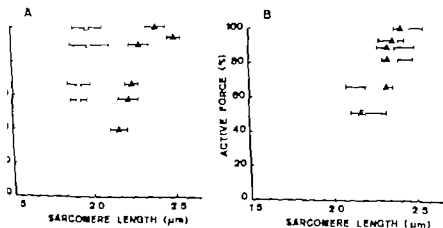


Fig. 3. Relationship between peak isometric force and resting sarcomere length in rat papillary muscles. Different symbols represent different muscles and each data point is the mean value of 5–30 measurements.



...chematic illustration of length seg... two papillary muscles (A and B) at ...ean contractions) and during activity ...of peak tension). Measurements from ...phic records of surface markers. ...s denote the distance between adja... ...kers and are expressed in percent of ...ting muscle length at L_{max}. The total ...ength was taken as the distance be... ...e points of attachment of the rigid ...o links that were used for mounting the ... Dashed lines combine corresponding ...s at rest and peak activity. The upper ...gment of the tracings contain the top ...the muscle and the tendon. Note the ...form behaviour of the different seg... ...long the length of the muscle as well as ... the two preparations.

...difference in behaviour of sarcomeres during activity is further illustrated in Fig. 5 A, ...ch presents data from two different muscles. In each muscle the mean sarcomere ...in selected region of the preparation has been determined at rest and during activity ...values at different overall lengths of the muscle are plotted against peak twitch force ...be seen in Fig. 5 A that the sarcomeres studied in this particular case shortened by



...5. Relationship between peak isometric force and resting (filled triangles) and active (open triangles) ...omere lengths measured in one region of two different papillary muscles (A and B). Note: 1. the similarity ...elationship between active force and resting sarcomere length in the two preparations and 2. the dis... ...ancy in sarcomere length recorded in the selected areas of the two muscles during activity.

approximately 0 35 μm as the muscle went from rest to peak activity at the various m
lengths. By contrast there was a slight elongation of the sarcomeres during activity a
muscle segment studied in the experiment of Fig. 5 B. As shown in Table 1 the ra
sarcomere length during activity to that at rest varied between 0.88 and 1.01 in the 7 mu
investigated

Stretching of the tendinous attachments of the papillary muscle could generally be
served during contraction In one of the muscles the collagenous fibrils in the tendon co
be clearly visualized. During rest the tendinous fibrils were arranged in a wavy pattern. W
the muscle contracted, the waves straightened out. This arrangement is likely to be
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Discussion

Resting sarcomere lengths in the living papillary muscle preparation are quite uniform a
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mum sarcomere length of 2.38 ± 0 004 μm was found, a value that is slightly though sig
cantly less than the 2.44 ± 0 01 μm of the present study ($p < 0.01$). Since basically the s
experimental approach was used in both studies, this difference is probably real. In
experimental design of the present experiment the muscles were to be repeatedly stret
and shortened Anticipating the possibility of some slippage (which, however could no
statistically demonstrated) we chose as our L_{max} (see Methods) that optimum length w
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between 2.4 and 2.5 μm In the previous study the minimum optimum length was cho
We feel that the most likely explanation for this difference between the previous and
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lengths

A great part of the present experimental results depends upon the integrity and stab
of our papillary muscle preparation. Since the muscle was tied via a block of ventric
muscle at one end and via the tendinous attachments at the other end the papillary mu
itself presumably was not damaged This assumption is supported by the normal microsc
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extreme care was taken not to touch the muscle with metal instruments nor to damage
muscle by overstretching nor to allow the muscle to shorten excessively After equilibr
the muscle at the given stimulation frequency the preparation was mechanically stable
many hours.

Sarcomere shortening as expected, occurred during the mechanical twitch. The fo
transducer s compliance (< 0.5%) resulted in only a very small part of the total Most of
compliance existed within the preparation. One source of the compliance was directly
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tening of length segments between external markers was less than the sarcomere short g; the endocardial layer (very thin in these animals) appeared to act as a sleeve over the ractile fibres. Sources of myocardial compliance, in addition to those mentioned above been suggested to be the interlocking cellular arrangement, the intercalated discs and Z discs. The magnitude of any of these sources is difficult to assess at the present time. he results showed that, though resting sarcomere lengths tended to be very uniform oughout the preparation, the extent of shortening during force development was quite able the sarcomeres in some areas were even found to elongate. It is tempting to speculate t the intertwining nature of the myocardial cells provides for the even distribution of se stresses placed upon them and that, in the relaxed state, this process is complete. ring activity the extent of shortening will depend upon the compliance seen by the indi- al contractile units and, apparently this compliance is quite variable within the papillary scle. This difference in local compliance need not reflect an uneven distribution of passive stic components within the preparation. The possibility exists that the contractile strength ies along the length of the fibre bundles in the muscle due to differences in cross-sectional a and/or regional differences in activation. This means that one region may shorten more nsively at the expense of weaker segments that act in series. The increased distribution of comere lengths during activity were also visualized by means of the laser diffraction tech- ue described by Cleworth and Edman (1972). The first-order diffraction pattern from the illary muscle broadened and became much less distinct as the muscle went from rest to activity Similar observations have been reported by Krueger and Pollack (1975) in dies of rat papillary muscles.

t is of interest to note that maximum force was obtained at substantially longer sarcomere gths (mean 2.44 μm passive length and 2.29 μm active length) than during tetanic contrac n in skeletal muscle. Active force was reduced to a low level (about 1/3 of the maximum) decreasing the sarcomere length to 2.0 μm, l to a length were tetanic force is maximum frog skeletal muscle. These findings agree well with results obtained by Krueger and llack (1975) in studies on rat papillary muscle and by Winegrad (1974) in experiments on z atrial preparations. However a quantitatively different relationship between active ce and sarcomere length has been reported by Julian and Sollins (1975) in rat papillary ncles; they report clearly shorter sarcomere lengths (both active and passive) at L_{max}. The observed difference between the length-tension relationships in cardiac and skeletal ncle are not readily explainable on the basis of a difference in length of the A and I fila- ents between the two preparations (Page 1974). A more plausible explanation takes account the fact that the cardiac length-tension curve refers to twitch responses rather than time lependent tetanic contractions. Evidence from previous studies on heart muscle (Edman d Nilsson 1968, Blanks 1970, Nilsson 1972, Allen, Jewell and Murray 1974) do suggest at both the intensity and the duration of activation are increased by increasing the length the preparation. Stretching the muscle to a longer length may increase the amount of tivator calcium that is released in response to the action potential (cf Edman and Kiessling 971). On this basis then the twitch amplitude may increase with increasing sarcomere length en hen exceeding the length at which the area of overlap between the A and I filaments is ximum (also see Close 1972). Conceivable mechanisms by which length may affect the

approximately 0 35 µm as the muscle went from rest to peak activity at the various lengths. By contrast there was a slight elongation of the sarcomeres during activity in muscle segment studied in the experiment of Fig. 5 B As shown in Table 1 the ratio sarcomere length during activity to that at rest varied between 0 88 and 1 01 in the 7 muscles investigated.

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A great part of the present experimental results depends upon the integrity and stability of our papillary muscle preparation. Since the muscle was tied via a block of ventricular muscle at one end and via the tendinous attachments at the other end the papillary muscle itself presumably was not damaged. This assumption is supported by the normal microscopic appearance of the sarcomeres at both ends of the preparation During surgical removal extreme care was taken not to touch the muscle with metal instruments nor to damage the muscle by overstretching nor to allow the muscle to shorten excessively After equilibration of the muscle at the given stimulation frequency the preparation was mechanically stable for many hours.

Sarcomere shortening as expected, occurred during the mechanical twitch. The force transducer s compliance (< 0 5 %) resulted in only a very small part of the total Most of the compliance existed within the preparation One source of the compliance was directly observed to be at the tendinous end and could be visualized in the microscope as a straightening of wavy collagenous fibrils Another source of compliance probably resides in the piece of the ventricular wall used for attachment of the muscle It should be noted that t e pe

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From the Department of Physiology University of Göteborg, Sweden

The Interrelation between Hypothalamically Induced Changes in Sympathetic Discharge to the Gastrointestinal and Cardiovascular Systems

By

Dick Delbro and Björn Lisander

Received 16 March 1977

Abstract

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In chloralose-anaesthetized, adrenalectomized cats, the sympathetically conveyed effects of topical hypothalamic stimulations on gastric volume, blood pressure and heart rate were systematically explored. The vagal nerves were cut but could be kept active by graded efferent stimulation. In the absence of such vagal activity hypothalamic stimulation had no appreciable influence on gastric volume, even though the stomach possessed considerable myogenic tone. When, however, vagal excitatory activity was present, hypothalamic stimulation could markedly affect gastric tone, indicating that the sympathetic fibres exert their inhibitory influence on the stomach *via* its cholinergic intramural neurons. Hypothalamic stimulations that caused reductions in pressure or heart rate also usually caused an enhancement of gastric tone. Similarly pressor responses were associated with decreases in gastric volume whereas stimulation-induced tachycardia was not linked to any particular type of gastric response. Thus, the hypothalamic sympathoinhibitory influences on the cardiovascular system seem closely connected to a suppression of the sympathetic outflow to the stomach. There is, on the other hand, no regular association between hypothalamic sympathoexcitatory influences on the cardiovascular and gastrointestinal systems. In fact, there is in many areas a suppression of sympathetic discharge to the stomach in association with cardiovascular stimulation.

It is well known that the sympathetic adrenergic fibres induce inhibition of gastrointestinal motility except in the sphincter regions. In most species, notably cat and man, the sympathetic fibres make their essential inhibitory connections with the cholinergic excitatory intramural neurons, with only insignificant influences on gastrointestinal smooth muscle cells directly (*cf.* Furness and Costa 1974). Consequently cholinergic excitatory activity has to be present if gastrointestinal motility should be used as an indicator of changes in the activity of the regional adrenergic fibre supply. Further the effects of vagotomy or of atropine administration have for such reasons to be interpreted with great caution in the identification of peripheral pathways mediating centrally elicited motility responses.

The stomach is a suitable organ for the study of sympathetic influences on the gastrointestinal tract. Following acute vagotomy there is hardly any activity in the cholinergic excitatory intramural neurons (Jansson and Lisander 1969). Therefore, it is experimentally possible to induce any desired level of "background" cholinergic activity in the stomach by

metabolism of activator calcium in the excitation-contraction coupling in cardiac muscl recently been discussed by Fabiato and Fabiato (1976)

Our sincere thanks for technical assistance are given to Mrs A. F Grimm, Mrs B Kronborg and C. Olsson

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or Artificial respiration was maintained with respiration pump. — In two expts. atropine [illegible] sulphate, Merck) or guanethidine (Ismelin Ciba) as given I — In all expts. the vagi [illegible] were dissected free in the neck, cut and arranged for graded stimulation in the efferent direction Grass S 5 stimulator to elicit increased gastric motility via the vagal excitatory fibres. The range of [illegible] parameters as therefore chosen as 0.5–10 Hz, 1 2 ms and 4–15 V.

[illegible] stimulation. The head of the animal was placed Horsley–Clark apparatus. A mono[illegible] stainless steel electrode was inserted into the hypothalamus. A constant current stimulator delivered [illegible] pulses of 1 ms duration and 0.1–0.3 mA amplitude (1–4 V). The frequency was varied within [illegible] (5–100 Hz). To identify the stimulation points, direct anodal current of 1 mA as passed [illegible] the electrode for 30 [illegible] each track and usually at H 2. The brain as then perfused th 10 [illegible]. Frozen sections, 50 μm thick, were cut and photographed under high magnification. The stimu[illegible] points were localised with reference to the end points of the tracks, the Horsley–Clarke coordinates [illegible] and the locations of the identified lesions. Each stimulation point in the individual expts. was then [illegible] to the nearest mm in the stereotaxic atlas of Snider and Niemer (1961). Thus, the error in localisa[illegible] of the points may amount to 0.5 mm.

Recordings of effector responses. *Gastric volume* as recorded ith large rubber balloon introduced [illegible] the oesophagus (for details see Jansson 1969). The intragastric pressure as in the individual expt. [illegible] constant, at level between 8–18 cm H_2O. The system was filled th body [illegible] ater and the [illegible] was connected to container placed on Statham strain gauge, recording the eight of the water [illegible] the stomach. The strain gauge was connected to Grass polygraph. In this way plethysmographically [illegible] changes of gastric volume could be continuously followed at largely constant transmural pressure. *[illegible] pressure* as recorded by Statham P 23 AC transducer connected to catheter in femoral artery. [illegible] rate was recorded on Grass tachograph unit, triggered by the arterial pulse curve.

Experimental procedure. I 17 cats, the interrelationship between the changes in blood pressure, heart [illegible] and gastric volume were studied I one and the same animal, up to three electrode tracks were stimu[illegible] at 50 Hz, 1 ms and 0.2–0.3 mA. The three tracks were L 1 2 and 3 in given frontal plane (A 10–14). [illegible] each track the electrode was lowered to H 6 and then stimulation as performed at every mm during [illegible] withdrawal of the electrode up to 2. Each of these points as stimulated in at least 3 animals, [illegible] and without simultaneous vagal activation. At the end of the series, 4 cats were systematically stimu[illegible] throughout the locations A 10–14, L 1 3, H 4 to −6 to compare in one and the same animal the [illegible] from [illegible] entral parts of the hypothalamus. The changes in heart rate and blood pressure to hypo[illegible] stimulation [illegible] ithout efferent vagal stimulation were compared to the changes in gastric volume [illegible] subsequent efferent vagal stimulation since this latter procedure is prerequisite to reveal ad[illegible] mediated changes in volume.

In 3 of he topical stimulations biphasic gastric responses were observed, i. with both positive [illegible] negative deflections. When this was the case, the largest deflection was used for the calculations ith [illegible] corresponding sign. Each stimulation lasted at least 30 but was always kept long enough to let the [illegible] develop fully. The maximum changes in stomach volume ere always compared to the maximum [illegible] creases, or decreases, in blood pressure and heart rate. If these two latter parameters were biphasic, [illegible] occurred only occasionally, the phase was used which corresponded in time to the maximal change [illegible] stomach volume.

Statistical treatment of data. The interrelationships between the changes in adrenergic fibre discharge [illegible] examined statistically on the basis that under the present experimental conditions an increased [illegible] volume implied an increased sympathetic discharge, as was the case with pressure or heart rate [illegible] creases. The procedure is best illustrated by an example. When it was to be determined whether, for [illegible] stance, blood pressure increases were predominantly related to decreases or increases in heart rate, the [illegible] number of pressor responses with tachycardia, on one hand, and on the other pressor responses ith brady[illegible] cardia were determined in each cat. These numbers were expressed in per cent of the total number of [illegible] stimulations performed in the individual animals. Then, the differences between these two percentages, [illegible] with their corresponding signs, were calculated for all the animals and used in Wilcoxon test at the 0.01 and 0.05 levels.

Results

I General character of the responses. As earlier described the vagi were cut and arranged for efferent stimulation of the gastric excitatory fibres in all expts. The hypothalamus could substantially influence gastric olume in either direction if stimulated against a background

graded efferent vagal stimulation, which is particularly advantageous in studies of the ce[illegible] or reflex involvement of the regional adrenergic nerve supply

Hypothalamic influences on gastric motility have been studied by several investigator[illegible] Thomas and Baldwin 1968) but fairly little interest has been devoted to the participation of sympathetic fibres *per se* in such gastric adjustments. One exception is the study by [illegible] Lisander and Martinson (1969) in cats where the attention was focused on some function[illegible] well defined hypothalamic regions, *i.e.* the defence area with adjacent pressor regions [illegible] the nearby sympatho-inhibitory area. Stimulation of the defence area induced a [illegible] adrenergic activation of the cardiovascular system, a cholinergically mediated vasodilat[illegible] in skeletal muscle and an activation of the adrenergic fibres to the stomach, expressed [illegible] as a volume increase The hypothalamic pressor responses, characterized by an [illegible] and vasoconstrictor fibre activation without any engagement of the vasodilator fi[illegible] likewise caused an increased adrenergic influence on the stomach, resulting in a vo[illegible] increase A topical stimulation of the hypothalamic sympatho-inhibitory area, on [illegible] hand led to a generalized inhibition of adrenergic fibre tone causing a fall in blood [illegible] and heart rate and a decrease in gastric volume. Thus, when these three hypothalamic [illegible] were stimulated the adrenergic fibre activity to the gastrointestinal tract was rep[illegible] altered in parallel with that to the cardiovascular system It should be stressed, how[illegible] that the sites thus studied represent only a minor fraction of the hypothalamic structu[illegible]

For such reasons the question arises whether a similar parallelism in sympathetic disch[illegible] to the cardiovascular and gastrointestinal systems is encountered also when other h[illegible] thalamic structures are stimulated. There are indeed, reasons to believe that this may [illegible] be the case mainly because there are such considerable differences at the medullary [illegible] spinal levels with respect to the organisation of the cardiovascular and gastrointest[illegible] adrenergic control. For example, the cardiovascular control is under a strong supras[illegible] excitatory influence implying that the activity in peripheral adrenergic "cardiovasc[illegible] neurons is drastically diminished following spinalization (*cf* Folkow and Neil 1971). [illegible] *sympathetic nerves to the gastrointestinal intramural ganglia, on the other* hand, form [illegible] efferent link in spinally mediated reflexes Their reflex activity is under a supraspinal mod[illegible] tion whose net influence is inhibitory in nature and the reflex excitability is therefore [illegible] enhanced following spinal cord transection (*cf* Furness and Costa 1974) Such differen[illegible] in the bulbospinal control of the cardiovascular and gastrointestinal adrenergic supply [illegible] to the possibility that their control may in important respects be differentiated also a[illegible] hypothalamic level. In the present paper a more systematic exploration was therefore [illegible] formed with respect to the hypothalamic influence on the adrenergic fibre discharge t[illegible] cardiovascular system and to the stomach, here representing the gastrointestinal system

Methods

The expts. were performed on 30 cats of either sex, weighing between 2.1 and 3.5 kg. The a[illegible] were deprived of food for 24 h before the expts. After induction with ether they were anesthetized [illegible] chloralose, 50 mg/kg b.wt. A tracheal cannula was inserted After midline laparot my the adrenals [illegible] approached transabdominally and ligated t eliminate the release of catecholamines t the blood st[illegible] Adrenocortical substitution was given by i. [illegible] jection of hydrocortisone (Solu-Gluc Erco, 10 mg/k[illegible] Gallamine triethiodide (Flaxedil May and Baker 4 mg/kg i) was used t [illegible] som tomot [illegible]



Cat 2.6 kg. Vagi cut and arranged for afferent stimulation (3 Hz, 1 ms, 4 V). Hypothalamic stimula- A13, L3, H 4 and 5. Note that the efferent vagal stimulation unmasks excitatory gastric responses thalamic stimulation (50 Hz, 1 ms, 0.2 mA). Thus, gastric excitation is here elicited together with as blood pressure and heart rate.

od pressure were more often associated with increases in heart rate (p 0.01) than any single type of heart rate response and vice versa.

adrenergically mediated changes in gastric volume were however not so well cor d to the changes in pressure and heart rate as these latter changes were mutually cor d. Only when depressor or bradycardia responses were induced, these cardiovascular tory responses were most frequently associated to decreases in gastric volume ($p < 0.01$), reflecting a generalized inhibition of adrenergic fibre activity to both the cardiovascular gastrointestinal systems. The hypothalamically induced reductions in blood pressure heart rate and in gastric volume were without exception slow in onset but could, in persist for several minutes after cessation of stimulation. Thus, the suppressed sym- tic activity regained its tonic activity first after such a long latency that a reverberating itory influence might be suspected.

situation was a different one concerning the hypothalamic points from which pressor tachycardia responses were elicited. Thus, blood pressure increases were linked to ses in stomach volume (p 0.01), indicating a differentiated sympathetic influence on cardiovascular and the gastrointestinal systems. Concerning the points eliciting heart increases, these responses were not significantly connected with any particular type of ric response (p 0.2). However in the majority of these hypothalamic points the direction he changes in sympathetic discharge to the cardiovascular and gastrointestinal systems opposite to each other which, as mentioned, was not the case when the sympathetic harge to the cardiovascular system was depressed.

the data, stimulations in the ventral hypothalamus were overrepresented (see Methods). further analysis, however revealed interrelations between the variables of essentially same character in these ventral parts as in the more dorsal regions.

...hical localization of response sites. Each hypothalamic point in this systematic as stimulated in 3–6 cats and the responses were averaged. A topographical

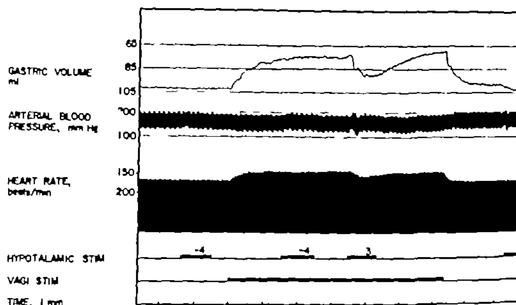


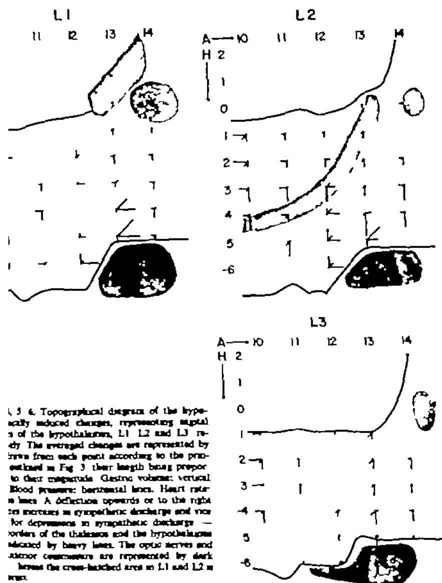
Fig. 1 Cat 2.2 kg. Vagi cut and arranged for efferent stimulation. Hypothalamic stimulation at 8 1 ms and 0.2 mA t A10, L1 H −4 and −3 Note the different character of the responses hen the elec is mo ed one mm and also that efferent vagal stimulation (2 Hz, ms, 8 V) is a prerequisite fo demonstration of the strong gastric inhibition at −3

of a prevailing efferent vagal stimulation but was virtually ineffective if activated a (see Fig. 1 and 2) The frequency-effector response relationships of these hypothal stimulations during efferent vagal stimulation at 1–4 Hz were studied in 5 cats. Increas stomach volume, reflecting an increased sympathetic discharge were maximal at 60–8 (3 points, 2 cats) whereas decreases in volume, due to inhibition of sympathetic act were maximal already at 40 Hz (5 points, 3 cats)

Pharmacological blockade of the gastric responses was performed in two cats. In animal the effect of guanethidine (4 mg/kg) was tested on the decreases in gastric volum hypothalamic stimulation which were blocked by this drug. In the other animal atro (1 mg/kg) was used which also blocked the decreases in gastric volume thus confirming observations and analysis earlier performed by Jansson, Lisander and Martinson (1969

2 *Interrelationships between cardiovascular and gastric changes* The hypothalamus systematically explored by topical stimulations in 17 cats. Altogether 464 points were sti lated and the sympathetically mediated changes in gastric volume blood pressure heart rate were recorded The interrelation between the changes observed are given in Table For each expt., the changes in the variables recorded at each stimulation were plo as follows Blood pressure versus heart rate blood pressure versus stomach volume heart rate versus stomach volume The data were then categorized according to the c stellation of changes and the number in each group in the table was expressed in relation the total number of stimulations in each cat on a percentual basis. In the Table are given averaged percentages in the various groups for all the 17 cats, together with the stand deviation.

The changes in heart rate were well correlated to those in blood pressure Thu



, 3 4. Topographical diagram of the hypo- ...ally induced changes, representing sagittal ...s of the hypothalamus, L1 L2 and L3 re- ...ely. The averaged changes are represented by ...drawn from each point according to the prin- ...outlined in Fig. 3 their length being propor ...to their magnitude. Gastric volume: vertical ...Blood pressure: horizontal lines. Heart rate: ...e lines. A deflection upwards or to the right ...tes increases in sympathetic discharge and vice ...for depressions in sympathetic discharge — ...orders of the thalamus and the hypothalamus ...ndicated by heavy lines. The optic nerves and ...anterior commissure are represented by dark ...between the cross-hatched area in L1 and L2 is ...fornix.

...ular function, in order to find out to what an extent they were linked together in a ...lar fashion when the various hypothalamic sites were excited. The mean values presented ...Fig. 4 therefore reflect both the direction and the average magnitude of the individual, ...pathetically conveyed responses within the cardiovascular and gastrointestinal systems ...they agree well in their general character with the stimulation responses in individual ...ts. with only few exceptions. They also confirm the Table insofar as depressor ...ycardia responses were regularly linked to decreases in gastric volume as were pressor

TABLE I The interrelationships between the cardiovascular and gastric changes, on a percental BP HR and SV denote blood pressure, heart rate and stomach volume, respectively. of a variable is indicated by the sign = 0, an increase by > 0 and a decrease by < 0 For information see text.

BP HR								
BP=0 HR=0	BP>0 HR=0	BP<0 HR=0	BP=0 HR>0	BP=0 HR<0	BP 0 HR>0	BP>0 HR<0	BP 0 HR 0	[illegible]
40.29 ±18.04	10.[illegible]4 ±11.15	10.29 ±6.66	0.49 ±2.01	1.24 ±3.81	15.91 ±16.2[illegible]	3.25 ±8.29	16.36 ±13.80	[illegible]
BP-SV								
BP=0 SV=0	BP>0 SV=0	BP<0 SV=0	BP=0 SV 0	BP=0 SV<0	BP>0 SV>0	BP>0 SV<0	BP 0 SV 0	[illegible]
34.47 ±14.65	7.06 ±10.22	6.56 ±7.61	1.14 ±[illegible].68	7.04 ±7.05	4.59 ±8.66	17.77 ±17.71	17.71 ±16.90	[illegible]
HR-SV								
HR=0 SV=0	HR>0 SV=0	HR<0 SV=0	HR=0 SV>0	HR=0 SV<0	HR>0 SV>0	HR>0 SV<0	HR 0 SV<0	[illegible]
42.09 ±13.53	2.19 ±4.20	3.79 ±6.04	2.01 ±3.94	17.35 ±11.90	5.41 ±9.15	10.65 ±12.97	14.53 ±12.69	[illegible]

representation of the effects of these stimulations is presented according to the princ outlined in Fig 3 Thus, *increases* in sympathetic discharge are indicated as 1) up- vertical deflections for gastric volume increases, as 2) upward oblique deflections for h rate increases and as 3) horizontal deflections to the right for blood pressure increases decreases in sympathetic activity the respective lines go in the opposite direction T length indicate the relative magnitude of the neurogenic effector responses. These princ are used for the construction of the response maps in Fig. 4 indicating the pattern of sponses obtained from the various hypothalamic sites at 1 mm, 2 mm and 3 mm latera the midline. These maps illustrate the main aim of the study *i.e* the exploration of relationships between the sympathetically mediated changes in gastric volume and in ca

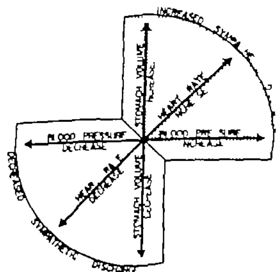


Fig 3 The principal arrangement of the sy used for the effector responses in the va point in Fig. 4 For further explanations text

in the absence of any prevailing vagal activity Moreover Jansson, Lisander and Martinson (1969) found that the alpha-adrenergic blocking drug, phenoxybenzamine, markedly ...ited the cardiovascular adrenergic responses to hypothalamic defence area and pressor stimulation but did not alter the gastric motility responses.

...the present expts., the carotid baroreceptors were left intact and their activity may have ... changed by the cardiovascular adjustments. However these receptors do not have any ...ficant reflex effects on the adrenergic outflow to the smooth muscle of the stomach wall ...son and Martinson 1966, Abrahamsson and Thorén 1972). Thus, it appears unlikely ...such reflexes should have contributed to the hypothalamically induced gastric responses.

...ly in 11% of the stimulations, there were cardiovascular changes without any con...itant alteration of gastric tone. This may simply mean that most hypothalamic structures ... influence the sympathetic nervous system produce changes both in cardiovascular and ...rointestinal functions. It is further obvious that topical stimulation may excite neuron ...ulations which are not necessarily normally involved in joint actions and such an error ...eases with rising stimulation intensity

...t is, however of greater interest to consider also the direction of the changes. With ...pect to depressor and bradycardia responses, indicating a reduction of sympathetic dis...arge to the cardiovascular system, this reduced discharge showed a close correlation to a ...uced sympathetic discharge also to the gastrointestinal tract ($p < 0.01$). The great majority ... blood pressure reductions noted in the present study were slow in onset, lasted for the ...ration of the stimulation, had ... long poststimulatory recovery period and were regularly ...ociated with reductions in heart rate. This is most characteristic of the sympatho-inhibi...y reaction (Folkow Johansson and Öberg 1959), where the efferent mechanism is an ...erall inhibition of sympathetic discharge t... both heart and vessels. It is interesting to note ...at these obviously sympathoinhibitory responses were so closely associated with signs of ...hibited sympathetic discharge also to the gastrointestinal system that it appears as if these ...o inhibitory components are normally closely coupled at the hypothalamic level.

The functional significance of such sympatho-inhibitory responses remains obscure. ...aving found that similar cardiovascular adjustments can be elicited from the anterior ...ngulate gyrus and that the hypothalamic sympatho-inhibitory area seems to serve as a ...ay station for these effects (Löfving 1961). Their resemblance to the circulatory changes ...duced by ... baroreceptor activation was noted and it was further suggested that the ...ypothalamic inhibitory area may form one of the links in a system mediating emotionally ...oloured responses such as fainting and the "playing dead" reaction met with in many ...pecies. H...ton and Spyer (1971) showed that destruction of the mentioned hypothalamic ...rea caused some impairment of the baroreceptor reflexes. Therefore, they envisaged the ...elay station for the baroreceptor reflexes as an elongated neuron pool, stretching from the ...ower medulla oblongata to include also these rostral hypothalamic structures. Even if this ...may be the case, it by no means denies that the bypothalamic sympathoinhibitory area may ...ell subserv... also other functions, such as "emotionally charged" response patterns. Further...more, the baroreceptor reflexes are normally devoid of any direct neurogenic influences on ...gastric motility (Jansson and Martinson 1966, Abrahamsson and Thorén 1972), while the ...hypothalamic sympathoinhibitory area has, ...part from in the present study been found to

responses, whereas tachycardia responses were not systematically associated wi particular type of gastric volume change

Points yielding cardiovascular changes only were not included in Fig. 4 They am to only 11 % of the whole material and the corresponding changes in heart rate and pressure were without exception minor The points were irregularly scattered with a te of being located to the outskirts of the autonomically active areas.

Discussion

The main aim of the present study was to examine the degree of parallelism in the changes of the adrenergic fibre supply to the gastrointestinal system and to the cardiova system in response to hypothalamic stimulation Heart rate and arterial blood pressure used as cardiovascular variables. As the vagi were cut and the adrenals eliminated, d in heart rate could only be explained by variations in adrenergic neurogenic tone. Fu increases in sympathetic discharge almost invariably cause increases of mean arterial p in cats, even in situations where the sympathetic cholinergic vasodilator fibres part in the overall response as in the defence reaction (*cf* Lisander 1970). Thus, in alm situations increases in pressure and heart rate indicate an augmented sympathetic dis and *vice versa*

Under the present experimental conditions, the hypothalamus could only influence volume *via* the sympathetic fibres and only if the vagi were simultaneously stimulated efferent direction In confirmation of earlier findings by Jansson Lisander and Ma (1969). They also observed that these neurogenic changes in gastric volume, augme as well as inhibitory were abolished by atropine or guanethidine, as confirmed in expts in the present series All these findings are compatible with an intramural, gan point of action of the adrenergic fibres with an inhibitory influence on the gastric chol neurons rather than on the smooth muscle effectors (*cf* Furness and Costa 1974).

The hypothalamically induced gastric responses were regularly associated with cha blood pressure and heart rate The question arises whether the changes in gastric v might be secondary to changes in gastric circulation. There is, however no clear relatio between gastrointestinal blood flow and motility (*cf* Furness and Costa 1974). Ko served, for example, that blood flow to the intestine had to be drastically reduced bef significant inhibition of motility occurred Carotid occlusion caused marked pres sponses but did not change motility in decentralized intestinal loops in cats with dene adrenals (Kock 1959) Thus, it appears less likely that changes in arterial pressure *per se* have caused any substantial gastrointestinal motility effects in the present This is particularly unlikely in view of the marked autoregulation of flow in the intestinal circuit tending to keep regional blood flow constant (*cf* Folkow and Neil A further strong argument is that the diencephalic stimulations affected gastric volum when a prevailing activity in the vagal excitatory fibres was maintained, despite the fac the gastric smooth muscle posesses a considerable myogenic tone even without such ef vagal stimulation In case neurogenic alterations in gastric blood flow affected the to gastric smooth muscle, one would have expected such indirect cha s in gastric vo

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cause sympathetically mediated decreases in gastric volume (Jansson, Lisander and M son 1969) and enhancements of colonic motility (Rostad 1973)

The question arises as to the mechanisms mediating the gastric effects from inh area stimulation. The present expts. involve an abdominal trauma which is known to ac spinal reflexes, collectively called the gastrointestino-gastrointestinal reflex, which pathetically conveyed and under the influence from higher CNS levels. Thus, it has a been found that these reflexes can be completely inhibited from supraspinal levels of nervous system (Johansson Jonsson and Ljung 1965 Martner 1975) It is therefore ter to assume that also the hypothalamic inhibitory area can exert such an influence, alth this problem requires further study

Pressor and tachycardia responses were obtained from extensive parts of the hypo and they were mutually closely correlated ($p < 0.01$) However pressor responses commonly occurring together with a decrease in gastric volume ($p < 0.01$). The same the case with tachycardia responses but here the association was not statistically signi ($p > 0.2$). These results strongly indicate that the hypothalamic sympathetic control m anisms of the gastrointestinal and cardiovascular systems are sometimes changed in pa but quite often also in opposite directions. In other words, the hypothalamic neuron seem to be organized in patterns that allow various types of combinations of efferent disch enabling the higher CNS levels to produce a variety of differentiated discharge pat

Most of the presently recorded pressor responses were encountered in medial and v parts of the hypothalamus, where according to Hess and Brügger (1943) aggressive beh is commonly elicited. The cholinergic sympathetic dilator fibres to skeletal muscle a excited from an overlapping zone, the defence area. Abrahams, Hilton and Zbrozyna induced patterns of flight or attack by stimulation of this area. There were some re differences with respect to the gastric responses associated with the presently observed p responses. Thus, in the anterior part of the pressor zone, gastric volume was usually dec suggesting a reduced sympathetic discharge to the gastrointestinal tract. More poste the pressor responses were associated with gastric volume increases, *i.e* here there wer signs of an increased sympathetic discharge to both the cardiovascular and gastroint systems. Again it may be so that, dependent on the nature of the environmental st and the associated emotional balance, various patterns of discharge may be elicited, times exciting, sometimes depressing gastrointestinal function in association with c vascular excitation in situations of fear rage *etc*

The present expts. have so far only aimed at examining the sympathetic effects on sto vessels and heart The vagal outflow to both heart and stomach may also be influence the hypothalamus (*cf* Lisander 1975), adding to the complexity of the system. It further be reemphasized that in the present expts. there has in all likelyhood b high degree of background reflex activity in the sympathetic nerves to the gastroint tract Expts. on non laparotomized cats may yield strikingly different results. also intended that the present results will be completed with studies on unanesthe spontaneously behaving animals, particularly to explore in which situations a gastroint excitation is associated with excitation of the cardiovascular system

This study was supported by grants from the Swedish Medical Research Cou 14X-4249) ... are due to Mrs Kerstin Bongsell for excellent technical assistance.

In an earlier report, Klevmark (1974) described a new cystometric method which was [illegible] to investigate the natural filling of the intact bladder. 5 different rate-models were [illegible]ested to study bladder behaviour during filling at physiological rates. At low filling [illegible] the pressure curve was always horizontal. At higher rates of filling a rise occurred in [illegible] intravesical pressure, and the new pressure level was retained when the filling rate was [illegible]bally reduced (pressure accumulation phenomenon). Results obtained using this [illegible] of cystometry were similar to those recorded during natural filling through spon[illegible]ous and stimulated urinary excretion.

[illegible]he purpose of the present work was therefore, to study the effects of extrinsic bladder [illegible]ervation on intramural tension (tonus) during controlled slow filling (Controlled Slow [illegible]tometry) and on intravesical pressure patterns during natural filling. The results show [illegible] the intravesical pressure reactions present in the intact cats are also found in the [illegible]ervated organ.

Methods

[illegible] and operational procedures. The results are based on data obtained from acute experiments on 35 cats of both sexes, weighing 2.3–4.3 kg.

[illegible] animals were anaesthetized [illegible] pentobarbital sodium (Nembutal, Abbott) [illegible] p. in doses of 30 mg/kg [illegible] small supplementary doses were given [illegible] as required. The animals were kept in the supine position [illegible] on [illegible] heated pad. *The rectal temperature* [illegible] maintained at about 37°C. *The blood pressure* was [illegible] with Statham pressure transducer by means of tubings inserted into the femoral artery. *Thoracic [illegible] movements* were recorded on the polygraph by [illegible] Grass volumetric pressure transducer type [illegible]5A. The animals [illegible] breathing spontaneously through [illegible] tracheostoma.

[illegible] reduce non-specific reactions due to surgical manipulations, exposure of the bladder to air and [illegible] to the initially injected volume, 30–40 min were allowed for adaptation, before recordings were [illegible].

[illegible] procedures. Through [illegible] low midline incision, the parasympathetic pelvic nerves lateral to the [illegible] were reached and sectioned central to their ramification. T[illegible] ensure radical denervation the closely [illegible] middle rectal artery [illegible] surrounding connective tissue were cut as [illegible]. Sympathectomy was [illegible] by section of the hypogastric nerves and removal of the inferior mesenteric ganglion. In expts [illegible] several hours the denervation procedures had to be done with special precautions. Denervation per[illegible] close to the bladder caused early bladder edema because [illegible] certain number of veins had to be [illegible], changing bladder [illegible] consistency and thus probably the resistance to stretch (compliance). Opera[illegible] performed too centrally on the pelvic or sacral nerves involved more extensive surgery which after [illegible] hours might result in [illegible] drop in blood pressure and in urinary excretion.

[illegible] procedures. Bladder filling was obtained through: (i) [illegible] constant-volume infusion pump, or [illegible] (ii) urinary excretion.

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After parasympathectomy [illegible] did not occur and in the expts. with spontaneous excretion of [illegible], the average observation time [illegible] as chosen as for the intact cat 7.5 h (Klevmark 1974).

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From the Institute of Neurophysiology University of Oslo, Norway

Motility of the Urinary Bladder in Cats during Filling at Physiological Rates

II Effects of Extrinsic Bladder Denervation on Intramural Tension and on Intravesical Pressure Patterns

By

BJORN KLEVMARK

Received 23 March 1977

Abstract

KLEVMARK B. *Motility of the urinary bladder in cats during filling at physiological ra... Effects of extrinsic bladder denervation on intramural tension and on intravesic... sure patterns* Acta physiol scand. 1977 *101* 176–184

The influence of extrinsic innervation on bladder wall tension (tonus) and on intravesical pressure was studied with a new cystometric proced re (Controlled Slow Cystometry CSC) and during filling. After parasympathectomy but not after sympathectomy the basal intra esical pres... ma kedly increased At low filling rate the partial or completely denervated bladder was filled fro... initial volumes without any increase in ntravesical pressure. At higher rates of f lling a pres... occurred, and this reaction was not affected by either parasympathectomy or sympathectomy A... I tact cat the new pressure level was retained when the fill g rat was gradually reduced. The intravesical pressure patterns observed duri g filling were preserved i the partial or comple... nervated organ. Thus, bladder adaptation during natural f lling occurs independently of extrinsic... tion.

Studies on regulation of intramural tension (tonus) during the collecting phase resulted in two opposite theories the myogenic and the neurogenic. According myogenic theory tonus reflects the physical properties of the bladder wall and is i... pendent on neural activity (Nesbit *et al* 1947 Tang and Ruch 1955 Kock and Po... 1963 Sabetian 1965 b a.o). The neurogenic theory refers to the accommodation bladder during filling as obtained by nervous inhibition, parasympathetic (Sherr... 1915 Langworthy and Kolb 1933 Kuru and Iwanaga 1966 a o) or sympathetic (Edv... 1968 a, Sundin and Carlsson 1972, De Groat 1975 a.o.). However in these repor... authors have been studying inhibition of the rise in intravesical pressure which is ob... during filling at high and non-physiological rates. The previous experimental metho... not necessarily illustrate bladder behaviour during physiological rates of filling

In an earlier report, Klevmark (1974) described a new cystometric method which was used to investigate the natural filling of the intact bladder. 5 different rate-models were tested to study bladder behaviour during filling at physiological rates. At low filling the pressure curve was always horizontal. At higher rates of filling a rise occurred in intravesical pressure, and the new pressure level was retained when the filling rate was gradually reduced (pressure accumulation phenomenon). Results obtained using this type of cystometry were similar to those recorded during natural filling through spontaneous and stimulated urinary excretion.

The purpose of the present work was therefore, to study the effects of extrinsic bladder denervation on intramural tension (tonus) during controlled slow filling (Controlled Slow Cystometry) and on intravesical pressure patterns during natural filling. The results show that the intravesical pressure reactions present in the intact cats are also found in the denervated organ.

Methods

Animals and operational procedures. The results are based on data obtained from acute experiments on 33 adult cats of both sexes, weighing 2.3–4.3 kg.

The animals were anesthetized with pentobarbital sodium (Nembutal, Abbott) i.p. in doses of 30 mg/kg. Small supplementary doses were given [illegible] as required. The animals were kept in the supine position lying on a heated pad. *The rectal temperature* was maintained at about 37°C. *The blood pressure* was recorded with Statham pressure transducer by means of tubings inserted into the femoral artery. *Thoracic respiratory movements* were recorded on the polygraph by a Grass volumetric pressure transducer type PT5A. The animals were breathing spontaneously through a tracheostomy.

To reduce non-specific reactions due to surgical manipulations, exposure of the bladder to air and filling to the initially injected volume, 30–40 min were allowed for adaptation, before recordings were started.

Denervation procedures. Through a low midline incision, the parasympathetic pelvic nerves lateral to the rectum were reached and sectioned central to their ramification. To ensure radical denervation the closely situated middle rectal artery and surrounding connective tissue were cut as well. Sympathectomy was performed by section of the hypogastric nerves and removal of the inferior mesenteric ganglion. In expts. lasting several hours the denervation procedures had to be done with special precautions. Denervation performed close to the bladder caused early bladder edema because a certain number of veins had to be ligated, changing bladder wall consistency and thus probably the resistance to stretch (compliance). Operations performed too centrally on the pelvic or sacral nerves involved more extensive surgery which after some hours might result in a drop in blood pressure and in urinary excretion.

Filling procedures. Bladder filling was obtained through, (i) a constant-volume infusion pump, or through (ii) urinary excretion.

Controlled Slow Cystometry (CSC) was used as described (Klevmark 1974). In short: A double-catheter was inserted into the vertex of the bladder through a lower midline incision. Intravesical pressure was recorded through a Statham pressure transducer connected to a Grass Model 7 Polygraph and calibrated to pressure changes of 0.5 cm H_2O. A Harvard multispeed constant-volume infusion pump was used for filling the bladder with saline at 37°C. *The rate-unit* was the calculated hour-diuresis (*HD*) of intact animals using 26 ml/kg/24 h as a normal value (1 HD = 1.1 ml/kg/h) (Spector 1956, p. 341). Thus, the normal rate of urinary excretion is the average rate during 24 h. *The filling rates* were in the physiological range of up to 15 times the normal rate (1–15 HD).

After parasympathectomy micturition did not occur and in the expts. with spontaneous excretion of urine, the average observation time was chosen as for the intact cat 7.5 h (Klevmark 1974).

Factors determining the intravesical pressure. The recorded intravesical pressure includes 3 factors (Kondo, Mitsuya and Clow 1974): (i) extravesical pressure (weight resting on bladder), (ii) intravesical hydrostatic pressure (weight of fluid above the catheter orifice) and (iii) pressure due to wall tension. The latter pressure factor is the only one which varies with the rate of filling during these expts. Extravesical pressure is constant when the animal resides in the same position and has free respiration (van Duyl [illegible] 1957, Klevmark 1974).

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The influence of extrinsic innervation on bladder wall tension (tonus) and on intravesical pressure p[atterns] was studied with a new cystometric procedure (Controlled Slow Cystometry CSC) and during [natural] filling. After parasympathectomy but not after sympathectomy the basal intravesical pressure [was] ma kedly increased. At low f ll ng rate the partial o completely denerv ted bladder [was] filled from nitial volumes without any increase in ntravesical pressure. At higher rates of filling a pressure [rise] occurred, and this reaction was not affected by either parasympathectomy or sympathectomy. [In the] intact cat, the new pressure level was retained when the f lling rate was gradually reduced. The intra esical pressure patterns observed duri g f lling were preserved in the partial or complete[ly de]nervated organ Thus, bladder adaptation during natural filling occurs independently of extrinsic [innerva]tion

Studies on regulation of intramural tension (tonus) during the collecting phase [have] resulted in two opposite theories the myogenic and the neurogenic. According [to the] myogenic theory tonus reflects the physical properties of the bladder wall and is [inde]pendent on neural activity (Nesbit *et al* 1947 Tang and Ruch 1955 Kock and Po[...] 1963 Sabetian 1965 b a.o). The neurogenic theory refers to the accommodation [of the] bladder during filling as obtained by nervous inhibition, parasympathetic (Sherr[ington] 1915 Langworthy and Kolb 1933 Kuru and Iwanaga 1966 a o) or sympathetic (Edv[ardsen] 1968 a, Sundin and Carlsson 1972, De Groat 1975 a o) However in these repor[ts] authors have been studying inhibition of the rise in intravesical pressure which is ob[served] during filling at high and non physiological rates The previous experimental metho[ds do] not necessarily illustrate bladder behaviour during physiological rates of filling.

In an earlier report, Klevmark (1974) described a new cystometric method which was to investigate the natural filling of the intact bladder. 5 different rate-models were used to study bladder behaviour during filling at physiological rates. At low filling the pressure curve was always horizontal. At higher rates of filling a rise occurred in intravesical pressure, and the new pressure level was retained when the filling rate was [illegible]ally reduced (pressure accumulation phenomenon). Results obtained using this [illegible] of cystometry were similar to those recorded during natural filling through spon[illegible]ous and stimulated urinary excretion.

The purpose of the present work was therefore, to study the effects of extrinsic bladder [illegible]rvation on intramural tension (tonus) during controlled slow filling (Controlled Slow [Cyst]ometry) and on intravesical pressure patterns during natural filling. The results show the intravesical pressure reactions present in the intact cats are also found in the [dene]rvated organ.

Methods

[Anim]als and operational procedures. The results are based on data obtained from acute experiments on 35 [adul]t cats of both sexes, weighing 2.3–4.3 kg.

[Th]e animals [w]ere anesthetized [w]ith pentobarbital sodium (Nembutal, Abbott) i.p. in doses of 30 mg/kg. Small supplementary doses were given [i.v.] as required. The animals were kept in the supine position [o]n [a] heated pad. *The rectal temperature* [w]as maintained at about 37°C. *The blood pressure* [w]as [mea]sured with Statham pressure transducer by means of tubings inserted into the femoral artery. *Thoracic [respi]ratory movements* were recorded on the polygraph by [a] Grass volumetric pressure transducer type [P]3A. The animals [w]ere breathing spontaneously through [a] tracheostomy.

[To r]educe non-specific reactions due to surgical manipulations, exposure of the bladder to air and [dis]tension to the initially injected volume, 30–40 min were allowed for adaptation, before recordings were [star]ted.

[Dene]rvation procedures. Through [a] low midline incision, the parasympathetic pelvic nerves lateral to the [rectum] were reached and sectioned central to their ramification. T[o] ensure radical denervation the closely [associ]ated middle rectal artery and surrounding connective tissue were cut as well. Sympathectomy was [perf]ormed by section of the hypogastric nerves and removal of the inferior mesenteric ganglion. I[n] expts. [last]ing several hours the denervation procedures had to be done with special precautions. Denervation per[form]ed close to the bladder caused early bladder edema because [a] certain number of veins had to be [liga]ted, changing bladder wall consistency and thus probably the resistance to stretch (compliance). Opera[tions] performed too centrally on the pelvic or sacral nerves involved more extensive surgery which after [som]e hours might result in [a] drop in blood pressure and in urinary excretion.

[Fill]ing procedures. Bladder filling was obtained through (i) [a] constant-volume infusion pump or [thro]ugh (ii) urinary excretion.

Controlled Slow Cystometry (CSC) was used as described (Klevmark 1974). In short, A double-[lumen cath]eter was inserted into the vertex of the bladder through [a] lower midline incision. Intravesical pressure [was] recorded through [a] Statham pressure transducer connected to [a] Grass Model 7 Polygraph and [sens]itive to pressure changes of 0.3 cm H_2O. A Harvard multispeed constant-volume infusion pump was [use]d for filling the bladder [w]ith saline at 37°C. *The rate-unit* was the calculated hour-diuresis (*HD*) of [the c]at (animals weighing 26 ml/kg/24 h as [a] normal value (1 HD = 1.1 ml/kg/h) (Spector 1956, p. 341). Thus, [the no]rmal rate of urinary excretion [is] the average rate during 24 h. *The filling rates* [w]ere in the physiological [r]ange of up to 15 times the normal rate (1–15 HD).

[After] parasympathectomy micturition did not occur and in the expts. [w]ith spontaneous excretion of [uri]ne, the average observation time was chosen as for the intact cat 7.5 h (Klevmark 1974).

Factors determining the intravesical pressure. The recorded intravesical pressure includes 3 factors ([T]erell and Clow 1974). (i) extravesical pressure (weight resting on bladder), (ii) intravesical hydrostatic [pr]essure (weight of fluid above the catheter orifice) and (iii) pressure due to wall tension. The latter pressure [fa]ctor [is] the only one [wh]ich varies with the rate of filling during these expts. Extravesical pressure is constant [w]hen the animal rests in the same position and has free respirations (von Garrelts 1957, Klevmark 1974).

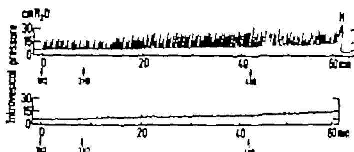


Fig. 1 Cat 2.8 kg. Intravesical pressure before (top) and after sympathectomy (bottom) at repeated controlled slow filling. Initial bladder volume 5 ml. Filling to the same final volume with increasing rates as indicated (1.3 and 4 HD) No rise in intravesical pressure was recorded at normal rate of filling (1 HD), but a gradual increase occurred at higher rates (3–4 HD). No change in intravesical pressure before and after sympathectomy Volume threshold for micturition (M) was not lowered. Note the change in spontaneous rhythmic activity following denervation (see text). The rate-unit (HD) 1 1 ml ... Methods).

Recording of the hydrostatic factor is reduced by short insertion and fixation of the catheter through top of the bladder Increase in hydrostatic pressure is kept constant during the expts. in the same animal filling to the same final volume. Thus, when the length (volume) factor of intramural tension (Laplace equation (Klevmark 1974)) is kept constant, a change in intravesical pressure represents a change in intramural tension.

Comment on the concept of tonus

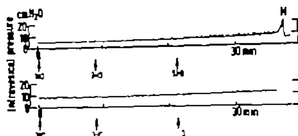
Intramural tension and tonus are identical concepts. However tonus is used more generally, intramural tension is usually restricted to walls of hollow organs. Difficulties exist in operationally defining tonus in mechanical terms. Theoretically comparison of pressure-volume or tension length measurements should indicate the degree of tonus reliably provided that the rate of distension is close to the physiological range of the actual organ (Alexander 1957). Bladder tonus has been operationally defined as resistance to stretch (Evans 1936) or as the slope of the pressure-volume curve obtained by cystometry (Tang and Ruch 1955) As already mentioned, the rate of distension has usually been outside the physiological range of the bladder Recently the term compliance has been proposed as substitute for bladder tonus, indicating the change in volume for change in pressure (Hald et al. 197

Results

Experiments described under (*A*) were performed to study bladder tonus in response to controlled slow filling before and after partial or complete denervation The bladder was filled from an initial volume of 5 ml to the same final volume before and after operation in the same cat. Filling was performed with infusion pump at rates between 1 15 HD through tubings inserted through the bladder top (see Methods) In (*B*) the intravesical pressure patterns demonstrated in intact cats were investigated after complete extrinsic denervation using natural filling through spontaneous and stimulated urinary excretion. The bladder was filled from an initial volume of 5–10 ml. Experiments in (*C*) were of methodological nature

A Effects of extrinsic bladder denervation on intramural tension (tonus) in response to controlled slow filling

(1) *Sympathectomy* Fig. 1 shows the intravesical pressure reactions in a typical cat before and after denervation during increasing rates of filling. In all 7 cats no rise in pressure



2 Cat 3 2 kg. Intravesical pressure before (top) and after parasympathectomy (bottom) in response to controlled slow filling. Initial volume 5 ml and filling to equal final volume with increasing rates (1.3 and 5 HD). No rise in intravesical pressure at normal rate of filling (1 HD), but a gradual increase occurred at higher rates (3–5 HD). After denervation the basal pressure was markedly elevated, but the rise in pressure at higher rates was unchanged. Micturition (M) was abolished.

observed at normal filling rate (1 HD). At higher rates of filling (3–4 HD in Fig. 1) the intravesical pressure gradually increased. However the intravesical pressure changes to the different rates of filling were identical in the intact and sympathectomized bladder. Volume threshold for micturition was not lowered. Spontaneous rhythmic contractions either disappeared or became irregular with low amplitude, and showed no significant response to stretch. The same changes of spontaneous rhythmic activity occurred after parasympathectomy and after complete denervation.

2) *Parasympathectomy.* At normal rate of filling (1HD) no rise in the intravesical pressure was observed in the intact or denervated bladder (Fig. 2). The pressure increases at higher rates of filling (3–5 HD in Fig. 2) were also identical in the two situations. In all 4 cats the basal pressure increased 2–4 cm H_2O after denervation. Micturition was abolished.

(3) *Complete denervation.* In all 3 cats which were parasympathectomized and sympathectomized no rise in basal pressure was observed (Fig. 3). No rise in intravesical pressure was observed at normal rate of filling (1 HD). At higher rates the pressure increase was more even as shown in Fig. 3. For the total observation time no overall difference in the pressure reactions was observed in the intact and completely denervated bladders. Micturition was abolished.

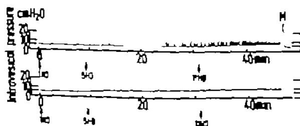


Fig. 3 Cat 3.4 kg. Intravesical pressure before (top) and after complete extrinsic denervation (bottom) in response to controlled slow filling. The initial volume was 5 ml and filling up to equal final volume was performed with increasing rates (1.5 and 11 HD). No rise in pressure was observed at normal rate of filling (1 HD). For the total observation time (0–45 min) no difference in total pressure rise was observed. Micturition (M) was abolished.

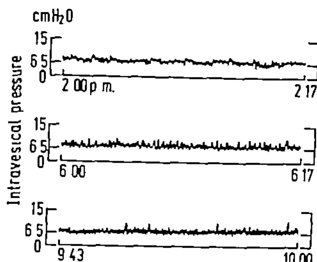


Fig. 4 Cat 2.1 kg. Intravesical pressure d... natural fill g at low rates (spontan... urinary excretion) after complete ext... denervation. The initial bladder volume... 10 ml and the tracings show 3 separate... periods. No increase in intravesical pres... during fill ng for 8 h with 26 ml of urine

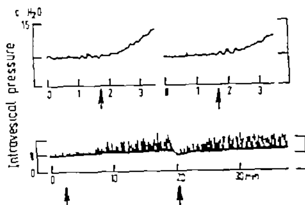
B *Effects of complete extrinsic denervation on intravesical pressure patterns during natural filling*

(1) *Filling by spontaneous urinary excretion* The intravesical pressure was recorded in... cats with an initial volume of 10 ml of saline. In 5 cats the arterial blood pressure (abo... 120 mmHg) and urinary excretion remained satisfactory during the experimental per... (2½ h to 9 h, average 7½ h) Average urinary excretion varied from 1–2 HD in 4 anim... and was ½ HD in one. No significant increase in intravesical pressure was observed, ... the micturition contraction was abolished after the parasympathetic denervation (Fig. ...

(2) *Filling by stimulated urinary excretion* In 4 cats with an initial bladder volume of ... ml of saline, glucose (5%) was administered i v at a rate of 20–25% of estimated blo... volume per h. In previous expts. this was found to give maximum rates of urinary excret... (15 times the normal rate) (Klevmark 1974) After 9–27 min the animals responded ... a smooth rise in the intravesical pressure which stabilized at a significantly higher le... 6–16 min after discontinuation of the infusion (pressure accumulation phenomenon) (Fig. ... Bladder volumes corresponded to an average filling rate of 4 4–9 3 HD suggesting ... considerably higher maximum rate of excretion. The intravesical pressure patterns observ... after complete denervation during spontaneous and stimulated urinary excretion we... identical to those previously described in the intact animal (Klevmark 1974).



Fig. 5 Cat 3.0 kg. Changes in intravesical pressure during natural filling t high rates (stimulated urin... excretion) after complete extrinsic denervation The initial bladder volume wa 10 ml. Urinary excre... was stimulated with intravenous infusion of 5% glucose (see text). Start of infusion at arrow to the l... A gradual rise in pressure began after 9 min (second arrow). The infusion was topped after 32 min (th... arrow). The rise in pressure continued for 16 min, thereafter the pressure stabilized at a higher level (fo... arrow) (pressure accumulation phenomenon). Final volume was 47 ml giving an average rate of urin... excretion of 9.3 HD



6. Intravesical pressure responses to repeated filling in the intact cat. Upper record: Cat 2.1 kg. ...al bladder volume ... at 10 ml. Filling (at arrows) to the same final volume with 10 ml at the rate of ... HD caused less increase in intravesical pressure the second time. Lower record. Cat 2.0 kg. Initial ...ume 10 ml. Filling (at arrows) to same final volume ...th 6 ml at the rate of 8.5 HD caused the same ...rease in intravesical pressure. Filling rate of 8.5 HD is within physiological range.

... Intravesical pressure responses to repeated fillings in the intact cat

...e following experiments were performed to study any possible reactions due to repeated ...etching of bladder wall tissue. In 5 cats repeated filling from the infusion pump to equal ...al ...olumes was performed using 10 ml of saline at 150 times the normal rate (150 HD). ... smaller rise in pressure was observed the second time (Fig. 6, upper record). This finding ...s to some degree inconstant when investigated from various initial bladder ...olumes. ...epeated filling to equal final volume using 6 ml of saline at the rate of 8.5 HD caused the ...me increase in pressure (Fig. 6, lower record). This was a consistent finding from various ...itial bladder volumes as long as physiological rates of filling were used.

Discussion

...egulation of intramural tension (tonus) during controlled slow filling

...the present work regulation of bladder tonus was studied employing a technique which ...ermitted the use of different physiological filling rates. When the rate of filling was low ...bout 1 HD), both the partially and the completely denervated bladder were filled without ...ressure increase as was the intact organ. After parasympathectomy the basal intravesical ...ressure was markedly elevated. However higher rates of filling gave intravesical pressure ...creases that were identical before and after sympathectomy or parasympathectomy.

The question of extrinsic nervous inhibition to counteract pressure increase at large ...ladder volumes (Edvardsen 1968 a, a.o.) has originated from cystometric studies using ...high and non-physiological filling rates. However the present study on denervated bladders ...as shown that, at large bladder volumes, there was no pressure increase to inhibit, as long ...s low physiological filling rates were used (Fig. 4). As in the intact cat (Klevmark 1974), ...ressure increase occurred at higher rates of filling, but at any volume. Thus, previous

cystometric methods, when used for the study of bladder tonus, do not illustrate bl behaviour in the true collecting phase

Sympathetic inhibition of bladder tonus has been postulated, either regulated t outflow from supraspinal levels (Gjone 1966) or through a spinal reflex (Edvardsen De Groat and Lalley 1972, Sundin and Carlsson 1972, Sundin, Carlsson and Kock However such inhibition was supposed to enhance the storage function by lowen intravesical pressure or bladder wall tension "at large volumes In the presen sympathectomy did not change the pressure increase obtained during higher physio filling rates irrespective of the volume present in the bladder

Parasympathetic inhibition of bladder tonus from spinal or supraspinal lev been suggested by several authors (Sherrington 1915 Langworthy and Kolb 1933 worthy and Hesser 1936, Kuru and Iwanaga 1966 a.o) On the other hand, Edv (1968 a) using conventional cystometry did not find efferent activity in the parasymp nerves during the collecting phase, findings that have been confirmed by De Gro Ryall (1969) This is also in accordance with the present results where parasympathe did not change the intravesical pressure rise during higher rates of filling. Howev basal intravesical pressure was markedly elevated after parasympathectomy This ph enon was not observed when the sympathetic nerve supply had been eliminated a Hence it can be tentatively explained by increased sympathetic nervous activity Exc *alpha*-adrenergic receptors have been located on postganglionic parasympathetic neur the bladder wall of the cat (De Groat and Saum 1972) Thus after extrinsic parasymp tomy this might be a site of increased sympathetic activity An apparent change β-adrenergic receptor response into an α-adrenergic receptor response has been d strated after parasympathetic bladder denervation in cats (Sundin and Dahlström Norlén *et al* 1976) Rise in intravesical pressure following parasympathectomy has reported earlier in cats (Langworthy Reeves and Tauber 1934) and in dogs (Langk Whiteside 1951) Carpenter (1951) in a histological study found bladder hypertrop cats where parasympathectomy had been performed and infection prevented. Yet Carpenter and Root (1952) did not observe bladder hypertrophy after parasympa denervation in the absence of residual urine. However the present observation in expts. of the elevated basal pressure with unchanged pressure increase (compliance) d filling has not previously been reported. As an observation performed under isom conditions only this might be interpreted as "hypertonicity"

Intrinsic or paravesical reflex activity accounting for regulation of bladder tonus also been postulated (Denny-Brown and Robertson 1933 b, Munro 1935). Howeve use of pharmacologic blocking agents has failed to demonstrate such activity (Nesbi Lapides 1948 Carpenter and Root 1951 Tang and Ruch 1955 Kock and Pompeius Taira, Matsumura and Hashimoto 1969)

After complete extrinsic denervation the pressure rise at higher rates of filling was cha during shorter periods (Fig. 3) This might be caused by the intrinsic short neuron scribed by Elbadawi and Schenk (1974) However it is difficult to see any import of this finding for normal regulation of bladder tonus.

In the present investigation, controlled filling was performed at increasing rates wi

physiological range. Such filling implied no measurable artifacts due to the procedure se (Fig. 6), thus making interpretation of effects of denervation more reliable and logically relevant. Results presented herein support strongly the theory of a non-rogenic basis of bladder tonus.

dder adaptation during natural filling

intravesical pressure patterns present during spontaneous and stimulated urinary retion in the intact animal (Klevmark 1974) were not changed by removal of the nomic nervous activity (Fig. 4 and 5). Thus, the "pressure accumulation phenomenon g 5) must be due to bladder wall properties. The shape of the pressure curve, the smooth and decline, is due to the gradual change in rate of urinary excretion present during ural filling.

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cystometric methods, when used for the study of bladder tonus, do not illustrate the behaviour in the true collecting phase.

Sympathetic inhibition of bladder tonus has been postulated, either regulated the outflow from supraspinal levels (Gjone 1966) or through a spinal reflex (Edvardsen 19… De Groat and Lalley 1972, Sundin and Carlsson 1972, Sundin, Carlsson and Kock 19… However such inhibition was supposed to enhance the storage function by lowering intravesical pressure or bladder wall tension at large volumes In the present… sympathectomy did not change the pressure increase obtained during higher physiol… filling rates irrespective of the volume present in the bladder

Parasympathetic inhibition of bladder tonus from spinal or supraspinal levels h… been suggested by several authors (Sherrington 1915 Langworthy and Kolb 1933, La… worthy and Hesser 1936, Kuru and Iwanaga 1966 a.o) On the other hand, Edvard… (1968 a) using conventional cystometry did not find efferent activity in the parasympathetic nerves during the collecting phase, findings that have been confirmed by De Groat… Ryall (1969) This is also in accordance with the present results where parasympathectomy did not change the intravesical pressure rise during higher rates of filling. However, t… basal intravesical pressure was markedly elevated after parasympathectomy This phenom… enon was not observed when the sympathetic nerve supply had been eliminated as w… Hence it can be tentatively explained by increased sympathetic nervous activity Excitatory *alpha*-adrenergic receptors have been located on postganglionic parasympathetic neurons… the bladder wall of the cat (De Groat and Saum 1972). Thus, after extrinsic parasympathec… tomy this might be a site of increased sympathetic activity An apparent change of… β-adrenergic receptor response into an α-adrenergic receptor response has been demon… strated after parasympathetic bladder denervation in cats (Sundin and Dahlström 19… Norlén *et al* 1976) Rise in intravesical pressure following parasympathectomy has be… reported earlier in cats (Langworthy Reeves and Tauber 1934) and in dogs (Langley… Whiteside 1951). Carpenter (1951) in a histological study found bladder hypertrophy… cats where parasympathectomy had been performed and infection prevented. Versus… Carpenter and Root (1952) did not observe bladder hypertrophy after parasympathetic denervation in the absence of residual urine. However the present observation in ac… expts. of the elevated basal pressure with unchanged pressure increase (compliance) during filling has not previously been reported. As an observation performed under isometr… conditions only this might be interpreted as "hypertonicity"

Intrinsic or paravesical reflex activity accounting for regulation of bladder tonus has also been postulated (Denny-Brown and Robertson 1933 b, Munro 1935). However t… use of pharmacologic blocking agents has failed to demonstrate such activity (Nesbit… Lapides 1948, Carpenter and Root 1951 Tang and Ruch 1955 Kock and Pompelus 19… Taira, Matsumura and Hashimoto 1969)

After complete extrinsic denervation the pressure rise at higher rates of filling was changed during shorter periods (Fig. 3). This might be caused by the intrinsic short neurons de… scribed by Elbadawi and Schenk (1974) However it is difficult to see any importance of this finding for normal regulation of bladder tonus.

In the present investigation, controlled filling was performed at increasing rates with…

Acta physiol. scand. 1977. 101. 185–193

From the Department of Zoophysiology, University of Göteborg, Sweden

Effect of Atropine and SC 15396 on Stimulated Gastric Acid Secretion in the Atlantic Cod, *Gadus morhua*

By

BJÖRN HOLSTEIN

Received 24 March 1977

Abstract

HOLSTEIN, B. *Effect of atropine and SC 15396 on stimulated gastric acid secretion in the Atlantic cod,* Gadus morhua. Acta physiol. scand. 1977. *101.* 185–193.

The acid secretion was measured in swimming codfish surgically equipped with a catheter draining the stomach. Gastric acid secretion was stimulated by histamine (5 or 15 μg/kg · h) or by carbachol (5 μg/kg · h). Pretreatment with atropine (1 μmol/kg · h) completely prevented the acid secretion induced by carbachol, but did not influence the secretion induced by histamine. Atropine had marked effects on the motor function of the stomach, and seriously reduced the volume draining from the stomach. Infusion of phenol red indicated that the decrease in volume was due to a decrease in recovery of ingested. After SC 15396, "antigastrin" significantly depressed acid secretion induced by histamine, and reduced carbachol-stimulated secretion, although the latter was statistically insignificant. The effects of SC 15396 is discussed with reference to the absence of receptors for gastrin related to gastric acid secretion in the codfish stomach.

Key words: Antigastrin, atropine, carbachol, fish physiology, gastric acid secretion, histamine

The codfish stomach responds to the i.m. injection of histamine or carbachol with the secretion of acid. Contrary to higher vertebrates, the codfish does not respond to pentagastrin, or to the injection of gastric mucosal extracts (Holstein 1975). This, together with the unpublished observation, that bombesin (a tetradecapeptide with gastrin-releasing properties) fails to induce gastric acid secretion in the codfish, suggests that receptors for gastrin are absent in the gastric secretory apparatus in this species.

In analogy with circumstances in many mammalian species, blocking of H_2-receptors with metiamide counteracts the stimulatory actions of histamine and carbachol (Holstein 1976). It remains an unsettled question whether this antagonism is due to some unknown property of metiamide, or to its H_2-receptor blocking capacity. In the latter case, at least two possibilities must be considered, either histamine is the final common chemostimulator of the acid secreting cell (Code 1956), or are the receptors for histamine and cholinergic agonists (and gastrin) interdependent, as suggested by Grossman and Konturek (1974). Supporting their hypothesis is the depression by atropine of histamine-induced acid secretion commonly found in mammalian systems.

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...e codfish stomach responds to the i.m. injection of histamine or carbachol with the secre... of acid. Contrary to higher vertebrates, the codfish does not respond to pentagastrin, ...to the injection of gastric mucosal extracts (Holstein 1975). This, together with the un...blished observation, that bombesin (a tetradecapeptide with gastrin-releasing properties) ...ls to reduce gastric acid secretion in the codfish, suggests that receptors for gastrin are ...sent in the gastric secretory apparatus in this species.

In analogy with circumstances in many mammalian species, blocking of H_2-receptors ...th metiamide counteracts the stimulatory actions of histamine and carbachol (Holstein ...76). It remains an unsettled question whether this antagonism is due to some unknown ...operty of metiamide, or to its H_2-receptor blocking capacity. In the latter case, at least ...o possibilities must be considered, either histamine is a final common chemostimulator ...the acid secreting cell (Code 1956), or are the receptors for histamine and cholinergic ...gonists (and gastrin) interdependent, as suggested by Grossman and Konturek (1974). ...upporting their hypothesis is the depression by atropine of histamine-induced acid secretion ...ommonly found in mammalian systems.

The present investigation was undertaken to further characterize the regulatory pr of the codfish stomach In particular the effect of an anticholinergic drug on hist stimulated acid secretion was considered important. It was also considered of inte evaluate the effect of 2 phenyl-2-(2-pyridyl)-thioacetamide (SC 15396) on stimulated s tion. This drug was introduced as an antigastrin (Cook and Bianchi 1967), but subs work indicated that it was unspecific. The current idea, that the gastrin receptor is la in the secretory device of the codfish suggested the use of SC 15396 in this investig

Materials and Methods

Surgical procedure Codfishes with a mean weight of 515 g (310-770 g) ere used, and ere prepared for collection of gastric effluence using a technique described in detail elsewhere (Holstein 19 Briefly a polyethylene catheter (PE 200, Intramedic) was implanted in the pyloric part of the sto and passed through the intestinal and abdominal walls and finally through the trap of the aquari fraction collector The water swallowed by the fish, mixed with the gastric secretions, as delivered the catheter and divided into one-hour fractions.

Operations were carried out during the a.m., and the experiments during the next day Before ex the animals were kept in storage aquaria, without food, for at least one week. The ater temperat 10 C.

During the progress of the tudy it became necessary to assess the recovery of ingested ater Th end, fishes were equipped with an extra catheter (PE 50), inserted through the stomach all, approx half way between esophagus and the pyloric sphincter Through that tubing, phenol red, dissolved i water was injected at a rate f 670 μg/h (0.62 ml/h).

Determination of acidity and ater recovery When possible, 2-ml aliquots of the effluent were titrated (Autotitrator assembly Radiometer Copenhagen) with 0.05 M sodium hydroxide to the pH o aquarium water In cases f smaller samples, the whole sample was titrated following addition of m distilled water to make 2 ml.

The concentration of phenol red in each hourly fraction was determined by reading the optical de (503 nm) of 200 μl liquots, acidified with 2.0 ml 0 1 M HCl. The recovery of phenol red was then calcul and considered to mirror the recovery of swallowed water and of secreted gastric acid.

Drugs. Atropine sulfate, Carbamylcholine chloride (Carbachol), Histamine dihydrochloride (S SC 15396 (Searle). SC 15396 was dissolved I 50 polyethylene glycol (mol.wt. 370–430).

All drugs were administered as i.m. injections at a rate of 0.62 ml/h using a Braun Perfusor IV lab Pump. Doses given f histamine and carbachol refer t the salts.

Statistics For each fish, the mean hou ly acid o tput d ring drug (or placebo) administration was calculated. Then the means of these values were calculated for treatment nd placebo groups respec nd compared by t-test (Programme ST1-07 t Statistic evaluation (two-sample test)) using a Texas In ments SR 52 calculator The difference was considered significant if a $P<0.05$ was ttained (two-t test).

Results

1 *Pretreatment with atropine* The injection of atropine 1 μmol/kg h during 3 h had no e on the acid secretion induced by the subsequent administration of 15 μg/kg h hista (Fig. 1 Table I) By contrast, atropine in the same dose completely abolished the secr effect of 5 μg/kg h carbachol (Fig. 1 Table II)

Higher doses of atropine was used in combination with histamine only Neither 5 25 μmol/kg h during 3 h was able to significantly alter the mean hourly acid output du the infusion of histamine (Fig. 2, Table I) However both atropine doses decreased unstimulated acid output (Fig. 2, Table III) It will be noticed from Fig. 2 (lower pa that also volume output decreased during atropine treatment ever w s not

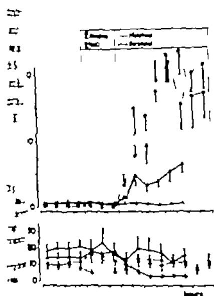


Fig. 1

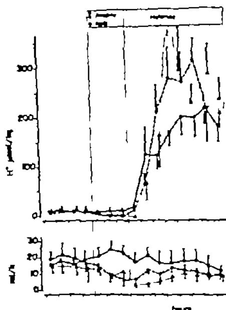


Fig. 2

Fig. 1. Effect of atropine (1 µmol/kg h) on gastric acid secretion stimulated by histamine (15 µg/kg h) or [illegible] (5 µg/kg h). Means and 1 S.E. Number of animals is given in Table I–II.

Fig. 2. Effect of atropine, 5 (●) and 25 ( ) µmol/kg h on gastric acid secretion stimulated by histamine, [illegible]/kg h. Means and 1 S.E. See Table I for number of animals.

[illegible] of the effect on unstimulated acid output, because in 12 of the 13 fishes, also the acidity [illegible]ased (not illustrated).

In Fig. 1 the results from 17 atropine-treated fishes are shown. In these fishes, atropine [illegible] no uniform effect on the volume output, but 4 additional experiments had to be dis[illegible]led due to ceased water flow when atropine was instituted. For the same reason, 9 fishes [illegible]ted with 5 and 25 µmol/kg h atropine were excluded.

Table I. Effect of pretreatment with atropine or BC 15396 on histamine-induced gastric acid secretion in the codfish

[illegible]	Dose µmol/kg h	Mean hourly acid output, µmol/kg h Mean ± S.E.	Number of animals	Significance
Atropine	0	145.8 ± 26.7	7	0.4 P NS
	1	120.7 ± 15.4	8	
Atropine	0	159.1 ± 18.2	7	
	5	199.8 ± 31.6	7	0.3 P NS
	25	202.7 ± 28.6	6	0.2 P NS
[illegible] 15396	0	120.8 ± 22.7	7	0.9 P NS
	10	118.4 ± 18.9	7	
[illegible] 15396	0	221.2 ± 30.4	7	0.001 < P 0.01
	50	93.0 ± 19.2	9	

TABLE II Effect of pretreatment with atropine or SC 15396 on carbachol-induced gastric acid se[cretion] in the codfish

Agent	Dose µmol/kg h	Mean hourly acid output, µmol/kg h Mean ± S.E.	Number of animals	Significance
Atropine	0	42.7 ± 6.8	10	P < 0.001
	1	5.5 ± 2.4	9	
SC 15396	0	61.3 ± 10.9	9	0.2 < P NS
	10	43.7 ± 6.7	10	
SC 15396	0	52.3 ± 6.9	10	0.1 < P NS
	50	33.9 ± 9.6	12	

Carbachol caused an immediate decrease in water output; this effect was prevented [by] atropine pretreatment (Fig. 1) confirming the suggestion that the decrease was med[iated] by muscarinic receptors in the stomach smooth muscles (Holstein 1976). In Fig. 1 ten [...] (saline–carbachol) fishes are reported, additionally 7 were discarded due to complete [...] of water flow during carbachol administration.

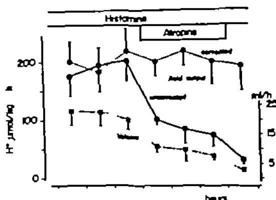
2. *Atropine during histamine injection.* In these experiments the fishes received 50 [...] histamine to initiate secretion and, at the same time, the infusion of 15 µg/kg h of hista[mine] was started. Atropine was given for 3 h, beginning after 5 h of histamine administra[tion]. Two doses of atropine were used, 2 and 5 µmol/kg h. The results were indistinguish[able] and are reported together. In 3 of the 17 fishes prepared for these expts., water output sto[pped] as soon as atropine was instituted. In the remaining, a more or less marked decrea[se in] water output took place and sometimes water flow stopped for one h, but was then res[umed]. From the measured parameters (decreasing volume and increasing acidity), a decre[ase in] acid output was calculated during atropine treatment (not illustrated).

The expt. was repeated on 18 fishes prepared for infusion of phenol red as describ[ed in] *Materials and Methods*. Of these, 12 immediately stopped delivering water when atro[pine] was injected, and 2 were apparently unaffected. In the remaining 4, the anticholin[ergic] caused a decrease in volume output, accompanied by an apparent inhibition of acid ou[tput] (Fig. 3). However, the recovery of phenol red also decreased, and when the acid output [was] corrected accordingly, no inhibition could be demonstrated.

The recovery of phenol red for the three h preceding atropine infusion was calculate[d]

TABLE III. Effect of atropine on unstimulated gastric acid secretion in codfish.

Mean hourly acid output during 3 h before treatment, µmol/kg h		Treatment Atropine µmol/kg h	Mean hourly acid output during 3 h treatment, µmol/kg h	
Mean ± S.E.	P		Mean ± S.E.	P
13.3 ± 3.4		0	14.2 ± 3.6	
10.7 ± 1.9	0.5 < P NS	5	3.1 ± 0.8	0.02 < P < 0.05
17.6 ± 6.2	0.5 < P NS	25	3.8 ± 1.2	0.02 < P < 0.05



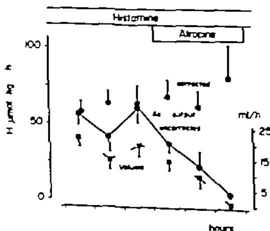
Effect of atropine (2 μmol/kg·h) imposed upon an injection of histamine (15 μg/kg h), on gastric acid secretion. h before atropine, 50 μg/kg histamine was administered to start the secretion. Phenol red was infused into the ch during the hole sequence. s and 1 S.E. (n 4)

hes (18) and found to be 91 4 ± 3.0, 86.1 ± 3 1 and 91 6 ± 1.8 (mean ± S.E.) for the h respectively

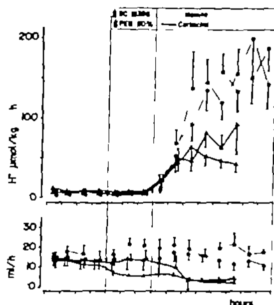
Qualitatively the same result was obtained with a lower dose of histamine, as shown in 4 Four fishes out of 9 maintained the delivery of water despite the fact that the atropine was reduced to 0.5 μmol/kg h.

Although the recovery of phenol red decreased when atropine was instituted, the concentration of the dye in the gastric effluence sometimes increased, sometimes decreased. s, the effect of atropine on water intake is inconsistent.

Pretreatment with SC 15396. Two doses of this drug were used. 10 and 50 μmol/kg h. lower dose had no effect on the histamine-induced acid secretion (Fig. 5 Table I), but reased the carbachol stimulated secretion to 71% of the control level. This effect, however, was not statistically significant (Fig. 5, Table II). The higher rate, 50 μmol/kg h, ed the histamine induced secretion to 42% of control level (Fig. 6, Table I) and the



4 See legend to Fig. 3. I these experiments, secretion was stimulated h 20 μg/kg plus 5 μg/kg·h histamine, ropine, 0 5 μmol/kg·h.



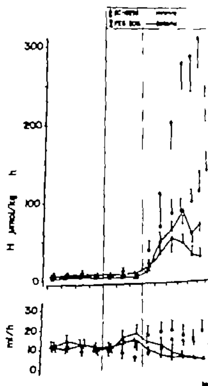


Fig. 5 Fig. 6

Fig. 5 Effect of SC 15396, 10 μmol/kg h, on gastric acid secretion stim lated by histamine (15 μ
o carbachol (5 μg/kg h). Means and 1 S.E. See Table I II for number of animals. PEG polyethylen

Fig. 6 See legend to Fig. 5 I these experiments, 50 μmol/kg h SC 15396 was infused.

carbachol-induced secretion to 65% (Fig. 6, Table II). Again, the effect on carb
induced secretion was not statistically significant.

4 *SC 15396 during histamine administration* When SC 15396 was superimposed up
infusion of histamine, the previously increasing acid output decreased (Fig. 7). SC-
had no effect on the water output, neither did it influence the recovery of phenol red,
was infused during these experiments

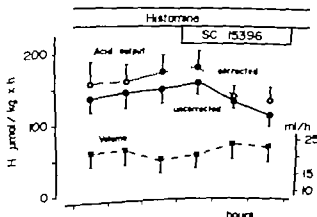


Fig. 7 Effect of SC 15396, 50 kg h, o gastric acid secretion stim by histamine, 15 μg/kg h. 5 h SC 15396, 50 μg/kg histamine jected. Phenol red was infused stomach during the whole se Means and 1 S.E. (n = 7)

Discussion

5396 ("antigastrin") is considered to be a general inhibitor of gastric acid secretion. and Thompson (1968) found that the drug antagonized the acid-stimulating effects of gastrin, insulin, and histamine. Kahlson *et al.* (1968) demonstrated inhibition of in- and histamine-induced secretion and concluded that the drug reduces the respons of the parietal cell to histamine. Such reduced sensitivity could be transmitted by a the antigastrin—as remarked by Tiongco *et al.* (1968)—provided that the activity of the in receptor is needed for full responsiveness of the parietal cell. However this view is to reconcile with the finding of Kahlson *et al.* (1968), that the gastrin-induced elevation histamine forming capacity is increased by SC 15396, and the demonstration by Caren (1969) and Håkanson and Liedberg (1971) that rat gastric histidine decarboxylase (Hd) treated by SC 15396. According to Håkanson and Liedberg (1971), Hd is stimulated by in which is released as a result of increased antral pH following SC 15396-blockade of secretion. Thus, the view that SC 15396 is a specific antigastrin can only be maintained receptors for gastrin related to gastric acid secretion are different from those mediating induction of Hd. Such differentiated specificity among receptors for gastrin was referred by Caren *et al.* (1969), as a possible explanation of the failure of secretin to counteract gastrin-induced activation of Hd.

The result of the present investigation confirms the conclusion that SC 15396 is an unspecific inhibitor of gastric acid secretion, because histamine-induced secretion was depressed. In a system devoid of gastrin receptors related to acid secretion, a specific antigastrin should *a priori* be without effect.

As expected, atropine is a powerful inhibitor of carbachol-induced gastric acid secretion in the cod. A dose-rate of 1 µmol/kg h (0.68 mg/kg h) during 3 h, completely abolished response to a subsequent 6 h-injection of carbachol. This rate, and even the 5- or 25-fold rate did not depress the secretion elicited by a subsequent injection of histamine. However evaluation of this result is complicated by the fact that some fishes stopped the delivery of water when atropine-treated. Thus, these fishes appeared to be more sensitive than the other concerning the motor activity of the stomach. This might also apply to the secretory once. When atropine was superimposed upon an infusion of histamine, the motor effects appeared exaggerated, but from few fishes decreasing volume output was maintained for some hours. The acid output during these hours decreased, but again the evaluation is complicated, this time by concomitant increase in acidity. The decrease in acid output reflects real inhibition if the increased H -concentration is the result of an atropine-induced reduction in drinking rate. It is equally likely however that atropine interferes with gut motility in a manner which decreases the recovery of ingested water. In that case, the decreased acid output is an artifact, resulting from loss of water and acid, presumably through the oesophagus. The latter interpretation is favoured by the results obtained with infusion of phenol red during atropine treatment there is a decrease in water recovery and following injection, no inhibition of histamine-induced secretion could be demonstrated. Admittedly decrease in recovery of phenol red could be the result of accumulation of fluid in a dilating stomach. If pooling takes place during atropine treatment, the corrected output values are

underestimated and therefore do not obviate the conclusion that no inhibition takes p
This demonstration, that acid secretion is unaffected by atropine despite concom marked effects on motor functions makes the results with atropine-pretreatment reliable, and it is concluded that in the codfish, histamine-induced gastric acid secre not amenable to inhibition by atropine.

In mammals, the inhibitory effect of atropine on histamine-stimulated secretion is competitive type (Konturek *et al* 1968 Borg and Emås 1970 Hirschowitz *et al.* 1972, In the present investigation two doses of histamine were used. Of these, at least one maximal, refuting the objection that a possible competitive inhibition was surmounted supramaximal dose of histamine

Grossman and Konturek (1974) when presenting the hypothesis of interdependent ceptors on the parietal cell postulate that, in the dog, blocking the acetylcholine re only weakly suppresses the histamine receptor whereas blocking the histamine re moderately inhibits the acetylcholine receptor" From the result of the present work, seems to be no reason to suggest any suppression of the histamine receptor when the linergic one is blocked. The reverse, however may still be true metiamide powerful tagonizes the stimulating effect of carbachol on gastric acid secretion in the codfish stein 1976)

The existence of a cholinergic link in the regulation of gastric acid secretion in the c is suggested by the stimulating effect of carbachol and by the blockade of that agonis atropine Also the basal secretion was depressed by atropine, but in that case, the site interaction is not necessarily peripheral It can not be exluded that basal secretion is by a mechanism which is centrally cholinergic, but peripherally non-cholinergic. Ho a vagal tone does not seem to be a prerequisite for atropine to inhibit histamine-stim acid secretion (Konturek *et al* 1968)

The finding that atropine is without effect on acid secretion stimulated by his indicates, that compared to mammals, the regulatory mechanism of the codfish se device is less complex. The result of a previous work in which pentagastrin was found ineffective as a secretory stimulant (Holstein 1975) also indicates a less complex or tion Whether this is a primitive condition, or a specialized one, is an open question.

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From the Department of Physiology, Gymnastik- och Idrottshögskolan, Stockholm, Sw

Fatigue and EMG of Repeated Fast Voluntary Contractions in Man

By

J. NILSSON, P. TESCH and A. THORSTENSSON

Received 24 March 1977

Abstract

NILSSON, J., P. TESCH and A. THORSTENSSON. *Fatigue and EMG of repeated fa[illegible] contractions in man.* Acta physiol. scand. 1977. 101. 194-198.

A fatigue test consisting of repeated fast maximal contractions of the left quadriceps muscle [illegible] apparatus was performed by [illegible] healthy male subjects (19-25 yrs). EMG signals were re[illegible] surface of the left vastus lateralis muscle, from which also biopsies were obtained for muscle [illegible] tion. Only minor changes were observed in the EMG variables despite a decrease in muscle [illegible] formance in terms of peak torque, work and power to about 50 % of initial values after 100 [illegible] The concomitantly obtained positive correlation between the increase in EMG/torque ra[illegible] dividual percentage of fast twitch (FT) muscle fibres indicated that local factors in the mu[illegible] in the FT fibres, were causing the development of fatigue during repeated dynamic contracti[illegible] power outputs.

Changes occurring in the EMG have often been used as a means to investi[illegible] mechanisms in human skeletal muscle. So far the studies have mainly been [illegible] fatigue during isometric loading. Still there are considerable disagreement [illegible] haviour of the EMG during fatigue. Merton (1954) found no changes in EMG [illegible] tained maximal contractions and therefore he ascribed fatigue entirely to th[illegible] processes in the muscle itself. In contrast Stephens and Taylor (1972) found [illegible] declined with fatigue during maximal voluntary isometric contraction. Durin[illegible] phase (about 1 min) the force fell to about 50 % and the IEMG decreased with th[illegible] course. In the second phase the force fell relatively faster than the IEMG an[illegible] approximately steady level at about 25 % of initial values. The authors conclude[illegible] muscular junction fatigue, mainly of the high threshold motor units, was mo[illegible] initially, whereas contractile element fatigue, mainly confined to the low thre[illegible] units, became more marked later. (High and low threshold motor units presum[illegible] pond to units with fast twitch, FT, type II, and slow twitch, ST, type I, musc[illegible] Burke and Edgerton 1975.)

Recently Thorstensson and Karlsson (1976) developed a fatigue test in whic[illegible] force was measured as peak torque during repeated maximal isokinetic contr[illegible]

[illegible] motion speed. Also in this test the force decreased rather rapidly to reach a plateau after [illegible] 60 s (corresponding to approximately 45 contractions). The decline in force was shown [illegible]sitively correlated to the proportion of FT fibres in the contracting muscle.

[illegible]s therefore felt of interest to apply an EMG analysis also on repeated fast dynamic [illegible]tions to get an indication about the fatigue mechanisms involved. In addition, the [illegible]ns between peak torque, power and work, as well as peak and integrated EMG were [illegible]ated at different levels of fatigue and with respect to the distribution of muscle fibre [illegible] the contracting muscle.

Material and Methods

[illegible] 12 habitually active male subjects volunteered for the study. Their mean age, height, and [illegible]eight [illegible]) were 22 ± 3 yrs, 180 ± 3 cm, and 70.3 ± 5.5 kg, respectively.

[illegible]procedure. The equipment and test protocol have been described in detail earlier (Thorstensson [illegible]976, Thorstensson and Karlsson 1976, Thorstensson 1976). Briefly, the subjects performed 100 [illegible]l extensions of the left knee joint [illegible]ith [illegible] constant angular velocity of 180 degrees/s ([illegible] 3.14 rad/s) [illegible]e of 50 contractions per minute. The range of motion was from 90 to 0 degrees knee angle, [illegible] each [illegible]ction lasted 0.5 [illegible] and the passive return approximately 0.7 s. The coefficient of variation for the [illegible] peak torque with 50 contractions [illegible]as 3.2 [illegible] (Thorstensson and Karlsson 1976).

[illegible]k torque was defined as the highest point on the torque curve.

[illegible]k was taken as the area under the torque curve and measured [illegible]ith [illegible] planimeter.

[illegible]er was calculated as work divided by the time for the contraction.

[illegible] means of contractions No. 1–3, 23–25, 48–50, 73–75, and 98–100 were taken for comparison. In all [illegible]ractions care was taken to ensure that the subjects did exert their greatest possible effort.

[illegible]cle *fibre classification*. Muscle biopsies were obtained from the vastus lateralis muscle with [illegible] needle [illegible] technique (Bergström 1962). Muscle fibres were classified as FT or ST after histochemical staining [illegible]yofibrillar ATPase (method originating from Padykula and Herman 1955).

[illegible]*G-recording*. The EMG activity was recorded from the contracting vastus lateralis muscle. EMG [illegible]ings from different portions of the engaged quadriceps muscle showed that the vastus lateralis was [illegible]entative for the [illegible]hole muscle group. Surface electrodes [illegible]ere used to pick up the activity. The ampli[illegible] EMG signals were rectified and filtered by [illegible] single lag filter of 120 ms time constant. The filtered [illegible]s were displayed on [illegible] UV-recorder (Honeywell Visicorder 2012) along with the corresponding torque [illegible]s.

[illegible]*k EMG* was defined as the highest point on the rectified and filtered EMG curve.

[illegible]*MG* [illegible]as taken as the area under the rectified and filtered EMG curve as measured by planimeter.

[illegible]istics. Ordinary statistical methods were used to calculate means, standard errors of the means [illegible]) and linear correlation coefficients (r). Differences were tested for significance [illegible]ith the Student's

Results

[illegible] values for peak torque, work and power averaged 140.8 N.m, 121.1 J and 272.2 W [illegible]ectively. Peak torque was well correlated with work (r [illegible] 0.80, p [illegible] 0.01) and power [illegible] 0.81 p [illegible] 0.001). A positive correlation (r [illegible] 0.75–81 p [illegible] 0.01) was also observed between [illegible] FT fibres and these variables during the initial contractions.

[illegible]eans ([illegible] S.E.) for peak EMG and IEMG during the initial contractions were 1.39 ± 0.11 [illegible] 1.26 [illegible] 0.11 in arbitrary units. The correlation coefficient between these measures was 0.93 (p [illegible] 0.001).

The changes occurring in the measured muscular force and EMG variables with increasing [illegible] fatigue are demonstrated in Fig. 1. Peak torque, work and power values declined [illegible] contraction. Thereafter these variables tended to plateau at a

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Recently Thorstensson and Karlsson (1976) developed a fatigue test in which the force was measured as peak torque during repeated maximal okinetic co

Discussion

results of the present study confirmed earlier findings with respect to the torque measure-s (Thorstensson and Karlsson 1976). Peak torque values declined progressively to reach ble level at about 50% of initial values after approximately 50 contractions. The relative se in peak torque was found to be related to the percentage of FT fibres in the con-ng muscle. Close agreement was found between peak torque, work and power in these rets.

owever despite the marked decline in torque no corresponding changes were observed e EMG. If anything, there was a slight increase in the EMG output during the first 25 ractions. One possible reason for this might be that the subjects did not exert maximal rts in the initial contractions of the fatigue test. To control this possibility an additional was performed under identical conditions. The subjects were asked to perform only ngle maximal efforts at the corresponding angular velocity (180 degrees/s). In this case measured variables tended to reach somewhat higher values (0–7%), but a statistically ficant difference (p 0.05) was seen only for work.

he present results were in contrast to those of Stephens and Taylor (1972), who found a ease in force during the initial phase of maintained maximal isometric contraction of first dorsal interosseous muscle of the hand that was of the same order of magnitude as or study. However they found a concomitant and proportional decrease also in the G, which pointed at the neuromuscular junction (NMJ) as the site of fatigue. Thus, ough the course of fatigue appeared similar the cause of fatigue seemed to be different he two experimental situations. Circumstances such as differences in the size and structure he muscles investigated, number and type of motor units recruited in maximal isometric l fast dynamic contractions as well as the intermittent character of the dynamic test have e considered when evaluating this discrepancy.

The present findings of an unchanged or even increased level of EMG activity with fatigue ld theoretically be explained by additional recruitment of motor units (with less ability produce force) as the number of active FT units decreased due to NMJ fatigue. However most likely explanation appeared to be that some local factor in the muscle itself was cause of the fatigue and not the neuromuscular junction. The correlation found between increase in EMG/torque ratio with fatigue and percentage of FT fibres indicated that detrimental factor for the decrease in force output was localized mainly in the FT fibres sing the successive drop out of this type of fibres. The positive correlation between the FT muscle fibres and the increase in lag phase between EMG activity and muscle force tput with fatigue would also be compatible with this hypothesis. Possible causes t fatigue ld be exhaustion of the elastic component, a lack of immediately available substrates and accumulation of metabolites, e.g. lactic acid. The latter possibility was supported by liminary results from lactate determinations on single muscle fibres in expts. with the me protocol (Tesch *et al* to be published).

study as supported by grants from the Swedish Medical Research Council (B75-04-4251-02) and the ounced of the Swedish Sports Federation.

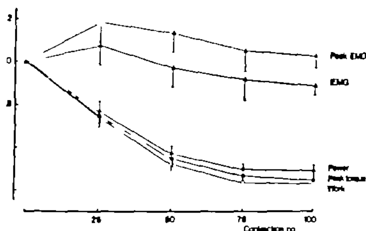


Fig. 1 Means + or SE measured variables during time course of fatigue The values were arbitrarily set (For absolute values, see ...

level corresponding to about 45–55% of initial values. Significant correlations were obs between relative decline in peak torque, work and power with 50 contractions and r = 0 75 ($p < 0.01$) r = 0 64 ($p < 0.02$) and r = 0 73 ($p < 0.01$), respectively

No corresponding decrease was observed in the EMG variables (Fig. 1). In contra peak EMG values for the 25th and the 50th contraction were higher ($p < 0.05$) the initial. A similar tendency (n.s.) was present for IEMG after 25 contractions. The chan EMG were not correlated to % FT

Both peak EMG per unit peak torque and IEMG per unit work demonstrated a pro sive increase from the initial to the 75th contraction, after which an apparent stead was reached. This level corresponded to about 2–2.3 times initial values. The relative crease in these ratios with 50 contractions was correlated to the individual percentage fibres. The correlation coefficients were r = 0 84 ($p < 0.001$ Fig. 2) and r = 0.78 (p respectively

The time lag between the first action potential in the EMG and the corresponding recording (= the point when the pre-set velocity was reached) increased progressively 95 ± 3 ms initially to 115 ± 5 ms at the 50th, and 121 ± 5 ms at the 100th contraction, re tively The initial values as well as the relative increase in time lag was correlated with (r = −0 68 $p < 0.02$, and r = 0.73 $p < 0.01$ respectively)

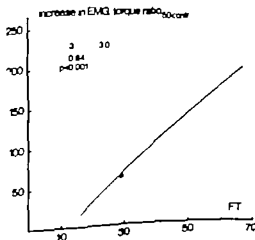


Fig. 2. Relationship between the relative increase peak EMG/peak torque ratio with 50 contractions and the percentage of FT fibres in the vastus lateralis mus 2).

Discussion

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Acta physiol. scand. 1977 101 199–210

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Frequency-Dependence of ^{3}H-Noradrenaline Secretion from Human Vasoconstrictor Nerves: Modification by Factors Interfering with α- or β-Adrenoceptor or Prostaglandin E_2 Mediated Control

By

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Abstract

[Stjä]rne, L. and J. Brundin. *Frequency-dependence of ^{3}H-noradrenaline secretion from human vasoconstrictor nerves: Modification by factors interfering with α- or β-adrenoceptor or prostaglandin E_2 mediated control.* Acta physiol. scand. 1977 *101* 199–210

...ted) superfused field stimulated human omental arteries and veins, preincubated with ^{3}H-(−)-nor[adren]aline (NA) were used to study the frequency dependence of NA secretion and of the mechanisms for local feedback control. ^{3}H-NA secretion per shock was found to be basically ... simple hyperbolic [func]tion of the stimulation frequency from 1 to 30 Hz, as long as secretion was restricted by prostaglandin [E$_2$] (PGE$_2$). In the absence of restriction, or during facilitation, ^{3}H-NA secretion per shock reached its [max]imum at 10 Hz and then declined at 30 Hz, indicating 'overload' in some link in the secretory mech[anism]. ^{3}H-NA secretion was depressed by exogenous NA and by PGE$_2$, and enhanced by isoprenaline, [phent]olamine and by blockade of PGE$_2$ formation. Most of these effects were inversely related to the [stim]ulation frequency. Attempts are made to study interactions between the different control mechanisms [and] to evaluate possible in vivo consequences of disturbance of adrenergic neuroeffector transmission by [inte]rference with the local control of the secretory mechanisms.

[Th]e amount of noradrenaline (NA) secreted from adrenergic nerve terminals, on arrival [of] propagated impulses, is not constant but subject to complex local control. It is [no]rmally restricted by an α-adrenoceptor mediated mechanism (Häggendal 1970, Farnebo [an]d Hamberger 1971, Kirpekar and Puig 1971, Starke 1972, Enero *et al.* 1972), and by [lo]cal formation of prostaglandin E$_2$ (PGE$_2$, Hedqvist and Brundin 1969, Hedqvist 1970, [Ju]tchison and Wennmalm 1971, Wennmalm 1972), and it may in certain tissues and [spe]cies be augmented by a β-adrenoceptor mediated mechanism (Adler-Graschinsky and [La]nger 1975, Stjärne 1975). These different control mechanisms have also been found [to] operate on the secretion of ^{3}H-NA from the adrenergic nerves of isolated human omental [b]lood vessels (Stjärne and Gripe 1973, Stjärne 1975 a, Stjärne and Brundin 1975 a, b, [19]76 a, b).

In many tissues and species the amount of NA secreted per impulse is also depe on the frequency of nerve stimulation (Brown and Gillespie 1957 for reviews see Ke 1975 Stjärne 1975 b) In a previous study of the relationship between the 'chem mediated local control and that exerted by variation in the frequency of nerve stim (in guinea pig isolated vas deferens), it was found that the efficiency of both the P dependent and PGE_2-independent α-adrenoceptor mediated control of ^{3}H-NA sec was inversely related to the frequency of nerve stimulation (Stjärne 1973).

In the abovementioned previous work concerning local control of the secre ^{3}H NA from the vasoconstrictor nerves of human omental blood vessels a freque 1 Hz was used, in order to ensure that stimulation was within the physiologically significant range. The present study was carried out to clarify the frequency depen of the process of H NA secretion in these nerves as well as the frequency depende the various mechanisms for its local feedback control

Material and Methods

The biopsy specimens (5–6 cm pieces of arteries and veins from the omentum, of an external diam bout 1 1.5 mm) were taken from 33 normotensive patients (30 female, 3 male, age range 16-6 median age 37) undergoing abdominal surgery for various causes, under routine general anesth most cases with halothane/oxygen/nitrous oxide/celocurine). Premedication always included a 0.5 mg, in addition to promethazine 25 mg or pethidine 25–75 mg s.c. In the 7 cases of Caesaream oxytocin (Syntocinon) 2 IU was administered i to the myometrium.

The method for labelling the neural stores with H-(−)-NA and for superfusion of the preparatio identical to that previously described (Stjärne and Brundin 1976 a). The electrode arrangement stimulation parameters were different however In the present study the intramural nerves ere stim via two platinum ring electrodes, one at the top and one at the bottom of the preparation (loop d 3 mm, distance between electrodes 5 cm). Stimulation was biphasic, and the shocks ere deliver Grass S44 stimulato at a voltage of 120 V and a duratio of 0.3 ms. During each stimulation per shocks were applied at frequencies ra ging from 1 Hz to 30 Hz.

Since the medium contained 0.6 μM desipramine and 10 μM normetanephrine, tissue binding ^{3}H NA should be minimized (Iversen 1971). The secretio of ^{3}H NA evoked by nerve stimulation a th expressed as Δt

$$\Delta t = \frac{\text{Evoked rise in efflux of } H}{\text{Calculated total } H\ l \text{ the tissue t beginning of stimulation}}$$

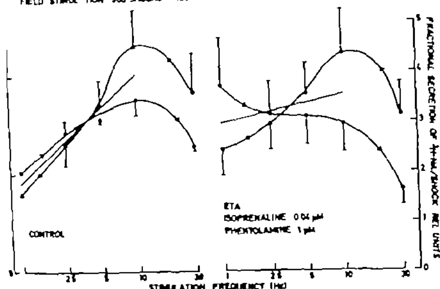
Nerve stimulations were applied with 16 (or d ring washes 3) ml intervals. Drugs, at the co centrations given in the paper were added to the medium 8 ml prior to the next stimulation The drugs did not alter the resting efflux of ^{3}H

The following drugs were used Isoprenaline (L-isoproterenol-D-bitartrate, Sigma), propranolol phentolamine (Hässle–Ciba–Geigy), noradrenaline bitartrate (Sigma), prostaglandin E_2 (Astr irreversible inhibitor of PGE_2 synthesis (Downing 1970) 5 8 11 14 eicosatetraynoic acid (ET received as a gift from Professor B. Samuelsson at the Department of Biochemistry Karolinska Ins

Statistical evaluation of the result was by Student t test. The f gures given in the paper re means ± S.E.

Results

A *Control values for Δt* The average absolute value for Δt per shock during the 2 3 control stimulations at 1 Hz applied at the start of each experiment was (5 63 ± 0. 10^{-4} (n = 122) of the total store of H NA All subsequent values for Δt obtaine stimulation either in the sequence 1 2.5 5 10 and 30 Hz, or l se or



1. Hysteresis in values for Δt on stimulation at rising or falling frequencies (1 30 Hz). Points and represent means and ranges of 2 observations. Regression lines show estimated slopes at 1 10 Hz. panel Δt in the absence of drugs (0.6 μM desipramine and 10 μM normetanephrine present through- *Right panel* Δt at highest degree of facilitation in the study. *Ordinates* Note differences between and right ordinate (same relative units, see the Text).

out addition of drugs, were normalized in relation to the average value for Δt during initial 'control' stimulations at 1 Hz in that same experiment.

Hysteresis in values for Δt In the absence of drugs Δt per shock increased with the quency of nerve stimulation, from 1 Hz to 10 Hz, but declined at 30 Hz, both when stimulations were applied in the order 1 2.5 5 10 and 30 Hz and when the sequence was rsed (Fig. 1 left panel). In spite of the considerable hysteresis illustrated in Fig. 1 re can be no doubt that Δt increased with the frequency of nerve stimulation from 1 to Hz, in the control experiments. The hysteresis was regarded as an indication of fatigue the secretory mechanism. Therefore only one series of frequencies was studied in each periment (after the 2 3 initial control stimulations at 1 Hz), either in the sequence 1 2.5 10 and 30 Hz or in the reverse order The results shown (except in Fig. 1) represent means S.E. of an equal number of experiments with rising and falling frequencies of nerve mulation. The rapid onset of 'fatigue' in the secretory mechanism, on stimulation in the sence of drugs very markedly raising Δt (Fig. 1 right panel shows the type of experi- ts here the highest levels of Δt were reached), made it very difficult to draw any liable conclusions from this type of approach concerning the effects of the drugs on the cretory mechanism at the different frequencies of stimulation. Because of this, additional periments were carried out where only 2 stimulations were applied after the initial 2 3

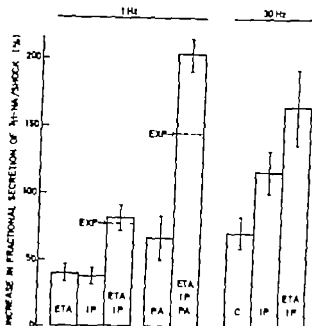


Fig. . Effect of drugs on Δt per sh stimulation for 2 periods (1 and 30 Hz of Results, B. Ordinate during control stimulation at 1 Hz ±S.E. Asterisks indicate $p<0.05$, $p<0.001$. Abbreviations: IP 0.04 μM. PA = phentolamine 1 μM expected. *Left panel:* Drug-induced at 1 Hz. ETA and IP alone: p controls. ETA + IP: p 0.01 to other or IP alone. PA: $p<0.001$ to ETA + IP + PA. $p<0.001$ to PA alone ETA + IP. *Right panel.* Stimulation Rise Δt over control at 1 Hz. Co 30 Hz. $p>0.05$ to control at 1 Hz. IP to control at 30 Hz. ETA + IP: p control at 30 Hz.

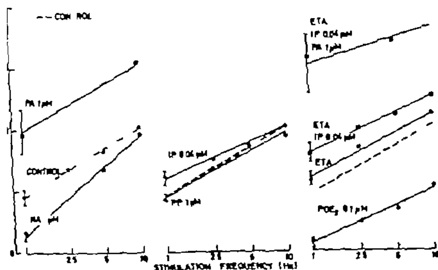
control stimulations, one at 1 Hz and one at 30 Hz, either with or without addition different drugs (Fig. 2).

C. *Frequency dependence of the secretory mechanism.* The values for Δt per shock ently increased with the frequency of nerve stimulation from 1 to 10 Hz, with or addition of drugs (Fig. 3), but declined on stimulation at 30 Hz (Fig. 1), except in ments where PGE was added to the medium (Fig. 4). This agent very depressed Δt per shock at all frequencies (see Fig. 3). The straight line obtained semilog plot of the PGE_2-depressed values for Δt as a function of stimulation freq from 1 to 30 Hz (Fig. 4) indicates that the efficiency of the secretory mechanism tended to increase hyperbolically with the total duration of depolarization per unit The decline in Δt per shock seen on stimulation at 30 Hz, in the other experiments the secretory mechanism was not depressed (control experiments, or experiments presence of drugs enhancing Δt) was therefore probably not due to failure of propa of nerve impulses at 30 Hz, but rather to beginning failure of some link in the sec mechanism (see also Fig. 6).

D. *Frequency-dependence of α-adrenoceptor mediated feedback control of* Δt. As Fig. 3 1 μM NA very markedly depressed Δt on stimulation at 1 Hz (by 67 1 ... p The depressing effect declined with increasing frequencies of stimulation (Fig. 5), an not statistically significant even at 5 Hz ($p>0.05$). Exogenous NA therefore change slope of the regression line shown in Fig. 3 (from 1 1013 for the control to 1.

Conversely 1 μM phentolamine appeared to enhance Δt at all frequencies (alth hysteresis caused a considerable scatter of values making significance evaluation diff and therefore did not tend to alter the slope of the regression lin howing the

FREQUENCY DEPENDENCE OF ³H-NA SECRETION IN HUMAN OMENTAL BLOOD VESSELS
EFFECTS OF INTERFERENCE WITH ITS FEEDBACK CONTROL
FIELD STIMULATION 300 SHOCKS 120 V 0.3 ms



Regression lines: Frequency-dependence of Δt in controls. Effects of drugs on frequency-dependence. Bars: Average S.E. for Δt at 1–10 Hz in controls or in the presence of drugs. Abbreviations: PA = phentolamine, IP = isoprenaline, PP = propranolol. *Left panel:* Effects of α-agonist and -antagonist. *Middle panel:* Effects of β-agonist and -antagonist. *Right panel:* Effects of exogenous PGE_2 and of ETA (to block formation of PGE_2). Effects of ETA, β-agonist or ETA + β-agonist, -antagonist. *Ordinate:* Same as in Fig. 1

endence of Δt in Fig. 3 (slope with phentolamine 1.0662). Phentolamine thus shifted regression line describing the frequency dependence of Δt per shock upwards by 73%. Relatively to control values at each frequency the enhancing effects of phentolamine on Δt were inversely related to the frequency of stimulation (Fig. 5). In the type of experiments mentioned under B (one stimulation period only at 1 and 30 Hz, Fig. 2) 1 μM phentolamine enhanced Δt at 1 Hz and 30 Hz by an average of 67.0% ($p < 0.001$) and 20.6% (p 0.05), respectively (n 8).

E. *Frequency dependence of β-adrenoceptor mediated control of Δt.* The effects of 0.04 μM isoprenaline on the frequency dependence of Δt represented practically a mirror image of those of NA (Fig. 3), although they were less marked. Isoprenaline changed the slope of the regression line in Fig. 3 to 0.8120 (control 1.1013). The significance study based on experiments of the type mentioned under B (Fig. 2) showed that this dose of isoprenaline enhanced Δt at 1 Hz by 37.7% ($p < 0.001$, n 12). Although the enhancing effect of isoprenaline on Δt declined with a rise in frequency (Fig. 3), it did in part prevent the decay of Δt per shock seen on stimulation at 30 Hz. Relatively to stimulation in the absence of drugs, isoprenaline enhanced Δt at this frequency by 26.5% (p 0.05, n 12). Thus isoprenaline differed from the other agents tested in having its maximal effects both at low and at high frequencies of stimulation (Fig. 5). Propranolol (1 μM) did not affect Δt at any frequency (Fig. 3).

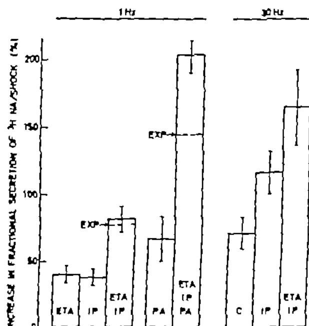


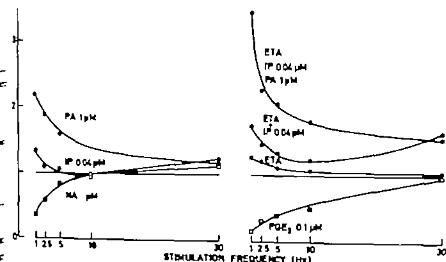
Fig. 2. Effect of drugs on Δt per [illegible] stimulation for 2 periods (1 and 30 Hz [illegible] cf. Results, B. *Ordinate* [illegible] during control stimulation at 1 Hz [illegible] ±S.E. Asterisks indicate p [illegible] p<0.001. Abbreviations: IP [illegible] 0.04 μM. PA = phentolamine 1 μM [illegible] expected. *Left panel*: Drug-induced [illegible] at 1 Hz. ETA and IP alone [illegible] controls. ETA+IP: p<0.01 to [illegible] or IP alone. PA: p [illegible] 0.001 [illegible] ETA+IP+PA: p<0.001 to PA [illegible] ETA+IP. *Right panel*: Stimulation at [illegible] Rise in Δt over control at 1 Hz. Control [illegible] 30 Hz: p>0.05 to control at 1 Hz. IP [illegible] to control at 30 Hz. ETA+IP: p [illegible] control [illegible] 30 Hz.

control stimulations, one at 1 Hz and one at 30 Hz, either with or without addition of different drugs (Fig. 2).

C. *Frequency dependence of the secretory mechanism.* The values for Δt per shock [illegible] ently increased with the frequency of nerve stimulation, from 1 to 10 Hz, with or without addition of drugs (Fig. 3), but declined on stimulation at 30 Hz (Fig. 1), except in [illegible] ments where PGE_1 was added to the medium (Fig. 4). This agent very mark[illegible] depressed Δt per shock at all frequencies (see Fig. 3). The straight line obtained [illegible] semilog plot of the PGE_1-depressed values for Δt as a function of stimulation frequ[illegible] from 1 to 30 Hz (Fig. 4) indicates that the efficiency of the secretory mechanism [illegible] tended to increase hyperbolically with the total duration of depolarization per unit [illegible] The decline in Δt per shock seen on stimulation at 30 Hz, in the other experiments [illegible] the secretory mechanism was not depressed (control experiments, or experiments [illegible] presence of drugs enhancing Δt) was therefore probably not due to failure of propa[illegible] of nerve impulses at 30 Hz, but rather to beginning failure of some link in the sec[illegible] mechanism (see also Fig. 6).

D. *Frequency-dependence of α-adrenoceptor mediated feedback control of Δt.* As [illegible] Fig. 5 1 μM NA very markedly depressed Δt on stimulation at 1 Hz (by 67.1%, p<[illegible] The depressing effect declined with increasing frequencies of stimulation (Fig. 5), a[illegible] not statistically significant even at 5 Hz (p>0.05). Exogenous NA therefore chang[illegible] slope of the regression line shown in Fig. 3 (from 1.1013 for the control to 1[illegible]

Conversely 1 μM phentolamine appeared to enhance Δt at all frequencies (alt[illegible] hysteresis caused a considerable scatter of values making significance evaluation di[illegible] and therefore did not tend to alter the slope of the regression line showing the freq[illegible]



5 Inverse relationship of the effect of drugs (exception: IP) to the frequency of stimulation. Ordinate: ... in the presence of drug divided by Δt for control at the same frequency. Only means are shown. *Left* ... Effects of adrenoceptor agonists and -antagonist. *Right panel*: Effects of PGE_2 and of ETA (to ... formation of PGE_2). Effects of ETA + β-agonist or of ETA + β-agonist + α-antagonist. The figure ... only data from expts with all frequencies 1–30 Hz (not those shown in Fig. 2)

this frequency the additional effect of ETA treatment was a further rise in Δt by 22.4% ... 0.05 n 23). Because of this the shape of the curve describing the relative effect of ETA + isoprenaline on Δt (Fig. 5) resembles that describing the effect of isoprenaline alone showing maximal effects at either low or high frequencies of stimulation, with a minimum ... stimulation at 10 Hz.

H *Effect of β-adrenoceptor stimulation after blocking α-adrenoceptors and PGE_2 formation*. Addition of 0.04 μM isoprenaline in the presence of 1 μM phentolamine, after infusion ... ETA, was found to cause rise in Δt to the highest levels reached in the study on stimulation at 1 10 Hz (Fig. 3). As shown in Fig. 1 the "fatigue" effects were so marked that the ... slope of the regression line also shown in Fig. 3 becomes of doubtful significance (its average upward shift, when compared to the control line, was by 126%). As shown in Fig. 2 the combined effects of all three drugs on Δt at 1 Hz were more than additive (expected rise as the result of isoprenaline + phentolamine + ETA 144.5%, observed rise 203.7). The effects of this combined treatment were highly significant, also when compared to the effects on Δt of ETA + isoprenaline (a further rise in Δt of 67.3%, p 0.001 ... 10) and to those of phentolamine alone (a further rise in Δt of 82.9 ..., p 0.001 n = 8). On stimulation at 30 Hz the combined treatment with isoprenaline – phentolamine + ETA showed an apparent rise in Δt per shock of 53.7% (about the same as with isoprenaline + ETA without phentolamine) when compared to the controls at this frequency (p > 0.2, due

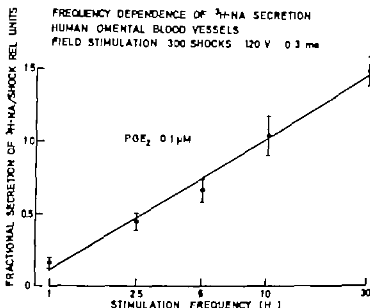


Fig. 4. Regression line [illegible] the frequency dependence [illegible] during depression of the [illegible] mechanism [illegible] PGE₂ [illegible] line in semilog plot [illegible] Δt was hyperbolic [illegible] the total duration of [illegible] tion per unit time. Mean [illegible] *Ordinate*: Same relative [illegible] ing Figs. 1 3.

F *Frequency dependence of PGE_2-mediated control of Δt* Exogenous PGE_2 at 0 [illegible] concentration depressed Δt on stimulation at all frequencies from 1 Hz to 10 Hz (b[illegible] at 30 Hz, see above under C and also Fig. 4) PGE_2 therefore depressed the regressi[on line] describing the frequency dependence of Δt by 61 8%, while only moderately altering [its slope] (from 1 1013 to 0 8610) The effects of PGE_2 on Δt on stimulation at 1–10 Hz were [illegible] significant ($p < 0\,001$ $n = 4$) Relatively to control values at each frequency the eff[ects of] PGE_2 were inversely related to the frequency of stimulation (Fig. 5)

Infusion of ETA (60 μM concentration for 10 min followed by washing) to block [illegible] tion of endogenous PGE_2 enhanced Δt significantly only on stimulation at 1 Hz. [illegible] expts shown in Fig. 3 infusion of ETA was found to cause a largely parallel upw[ard shift] of the regression line describing the frequency dependence of Δt by 15 0% without a[illegible] its slope (1 0034 as compared to 1 1013 for controls) In the experiments shown in [illegible] ETA was found to enhance Δt on stimulation at 1 Hz, by an average of 39 9% (p [illegible] $n = 11$) Relatively to control values for Δt the effects of ETA were inversely related [to the] frequency of stimulation (Fig. 5)

G *Effect of β-adrenoceptor stimulation after blocking PGE_2 formation* The eff[ect of] 0 04 μM isoprenaline were very markedly enhanced after previous infusion of E[TA to] block formation of endogenous PGE_2 (Fig. 3) The regression line was shifted up [illegible] from the control level by an average of 39 4%, on stimulation at 1–10 Hz, and the [illegible] was somewhat depressed (0 8821 against 1 1013 for the control) The results sho[wn in] Fig. 2 indicate that the effects of this combined treatment were purely additive, on sti[mula-] tion at 1 Hz. While ETA or isoprenaline alone caused a rise in Δt by 39.9% and 3[illegible] respectively the combined treatment was found to cause a rise in Δt of 81 5% (when com- pared to the effect of ETA alone $p < 0\,01$ $n = 11$) As expected from the enhancing eff[ect of] isoprenaline alone on Δt at 30 Hz the values for Δt at this frequency in the prese[nce of] isoprenaline and after ETA, were increased by 54 8% (Fig. 2) when compared to co[ntrols] at the same frequency ($p < 0.05$ $n = 11$) When compared to the effect of isoprenaline

nce of drugs strongly facilitating the secretory process (compare the moderate drift t values during unfacilitated secretion, in the left panel of Fig. 1 with the very rbing hysteresis during maximal facilitation, right panel). The accuracy of the Δt s obtained is therefore inversely related to the degree of facilitation. In order to test tatistical significance of the results separate experiments were carried out in which the uration was stimulated only twice (after the 2 3 initial control stimulations at 1 Hz), at owest and highest frequencies used in the study (1 and 30 Hz), in the absence or presence e various drugs. The moderate scatter of data thus obtained at 1 Hz makes these ts quite reliable, while the considerable scatter even with this approach makes it more ult to correctly evaluate data at 30 Hz.

Basic frequency dependence of the secretory mechanism: In agreement with results ned in many other tissues from several species (see Kirpekar 1975 Stjärne 1975 b) it found that the amount of neurotransmitter secreted per shock from the human motor nerves under study was highly dependent on the frequency of stimulation (cf hes 1972). Also in agreement with results from other tissues, the frequency-dependent Δt in the amount of transmitter secreted per shock, from the human vasomotor nerves, ed to decline at frequencies higher than 10 Hz and particularly when drugs were added h facilitated NA secretion (Haefely *et al.* 1965, Kirpekar and Misu 1967 Davies and rrington 1968, Hedqvist and Stjärne 1969 Stjärne 1973). One possible explanation for decrease in transmitter secretion per shock at 30 Hz is a decline in the size or number of ulses reaching the terminals (Kirpekar 1975), on stimulation at this relatively high uency However the finding that Δt per shock in the present work became a simple rbolical function of the total duration of depolarization per unit time, throughout a uency range from 1 Hz to 30 Hz, when secretion was depressed (with PGE_2, Fig. 4), es it likely that the fall in Δt per shock at 30 Hz, during normal or facilitated secretion due to failure in some link in the secretory mechanism, rather than to failure in con- tion of nerve impulses This is further supported by the similar findings of Kirpekar *al.* (1975), that the tendency to a decline in NA secretion per shock at frequencies higher 10 Hz, in phenoxybenzamine-treated cat spleen, was largely prevented when the secre was maintained at low level by reducing the calcium concentration in the medium.

Frequency dependence of α-adrenoceptor mediated control of Δt Both the effects of α-agonist NA and of the -antagonist phentolamine were found to be inversely related he frequency of stimulation. However there was a marked difference in their degree of uency dependence, reflected in the effects on the slope of the regression lines shown in 3 The fact that the inhibitory effects of 1 μM NA were apparent only at the lowest uencies of stimulation need not imply that the secretory mechanism is not sensitive to inhibitory effect of NA, on stimulation with frequencies of 10 Hz or higher It could tead imply that stimulation at the higher frequencies leads to accumulation of endo- ous NA to concentrations higher than 1 μM (neuromuscular gap in human omental od essels 30-100-500 nm, Thureson-Klein *et al.* 1976, *cf* Bevan and Su 1974). Con- ent with this is the finding that the enhancing effect on Δt of the competitive antagonist ntolamine declined more slowly with rise in stimulation frequency (Fig. 5), but ceased ficant at 30 Hz.

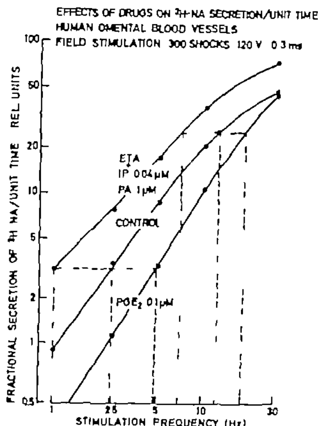


Fig. 6. Change in frequency req... balance facilitation (ETA + IP + PA... pression (PGE₂) of Δt per shock. F... see the Text.

to the wide scatter shown in Fig. 1) The relative effect of the combined treatme... isoprenaline phentolamine and ETA was therefore inversely related to the frequ... nerve stimulation (Fig. 5)

I *Effects of drugs on the frequency dependence of Δt per unit time* Since the decisi... in neuro-effector transmission is the concentration of neurotransmitter at the leve... postjunctional receptors, the values for Δt per unit time were calculated and plotted... the stimulation frequency (Fig. 6) comparing the control curve to those o... during the highest degree of facilitation (ETA + isoprenaline + phentolamine), or inl... (PGE_2) of the secretory mechanisms observed in the study As shown in Fig. 6, a ... rise in frequency would be required for the 'unfacilitated control transmitter secret... unit time to match that reached on stimulation at 1 Hz under maximal facilitation. ... PGE_2-mediated depression of the secretory mechanism a 5-fold rise in frequency... 1 to 5 Hz, would be required to reach the same level. At higher frequencies the diff... decrease To attain the value for Δt per unit time obtained on stimulation ... facilitation with ETA + isoprenaline + phentolamine at a frequency of 7 Hz, the ... stimulation in the absence of facilitation would have to be raised by a factor of 1 8 ... Hz, and in the PGE_2-depressed state the frequency would have to be increased to 18 5 Hz.

Discussion

A *Methodological difficulties.* The main difficulty was the rapid onset of 'fatigue ... secretory mechanism on serial stimulation at 1 2.5 5 10 and 30 Hz. In particular

ce of drugs strongly facilitating the secretory process (compare the moderate drift
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entolamine declined more slowly with a rise in stimulation frequency (Fig. 5), but ceased
o be significant at 30 Hz.

D *Frequency dependence of β-adrenoceptor mediated control of Δt:* The enhanc of 0 04 μM isoprenaline on Δt, on stimulation at 1 Hz, was only about 38%, as co to 68–69% found in earlier studies of the same type of preparation (Stjärne and B 1975 b, 1976 a) This difference may be due to the electrode arrangement and sti parameters used in the present study which also resulted in much higher control v Δt, during the initial stimulation periods at 1 Hz (an average of 5 63 10^{-4} as comp 2.45 × 10^{-5} to 4 09 10 of the ³H NA in the tissue at the time of stimulation, in t earlier studies) This may suggest that the effects of exogenous current and of tion of β-adrenoceptors were in part directed against the same target in the mechanism.

In agreement with earlier observations (Stjärne and Brundin 1975 b, 1976 a) pro did not markedly affect Δt, at any stimulation frequency This indicates that the mechanism in these nerves was not to any important extent dependent on β-adre mediated facilitation (Stjärne and Brundin 1975 b), as proposed by Adler-Gra and Langer (1975) for various tissues.

Isoprenaline was unique in tending to enhance Δt both at low (1 Hz) and (30 Hz) frequencies. The effect at 30 Hz was not statistically highly significant (Fi together with the fact that ETA and isoprenaline in combination significantly Δt at 30 Hz (where ETA by itself was without effect) it indicates that β-adrenocepto tion sustained the secretory capacity on stimulation at higher frequencies.

E. *Frequency dependence of PGE_2-mediated control of Δt* The extreme inhibitory of exogenous PGE previously demonstrated in human vasoconstrictor nerves on tion at 1 Hz (Stjärne and Gripe 1973 Stjärne and Brundin 1976 c) was verified to operate also at 2.5–10 Hz, although with decreasing efficiency at 30 Hz PGE affect Δt. The same inverse relationship to stimulation frequency was observ enhancing effect on Δt of blockade of formation of endogenous PGE_2 with ETA The effect of ETA on stimulation at 1 Hz in the present study was somewhat lo that observed with the electrode arrangement and stimulation parameters previo (39 9% as compared to 49 2% in the work of Stjärne and Brundin 1976 b).

F *Interaction between the different control mechanisms.* The combined effect of iso and ETA was additive, at least at 1 Hz (Fig. 2) and possibly reached its 'ceiling and Brundin 1975 b, 1976 b, work to be published). $β_2$-adrenoceptor mediated fa of Δt may thus be antagonized by endogenous PGE_2, although probably not by co for the same target in the secretory mechanism (Stjärne and Brundin to be p

The combined effect of ETA isoprenaline and phentolamine was more than (Fig. 2). This indicates that the α-adrenoceptor mediated inhibitory effect of en NA on Δt is not or is only in part, directed towards the same target as that of the β ceptor mediated facilitation of the secretory mechanism. Work is in progress to an interaction in more detail

G *Functional effects of interference with feedback control of Δt* The preser support those previously reported for several other tissues from various species co complex local control of the amount of NA secreted from adrenergic terminals, o of propagated nerve impulses. The efficiency of electro-secreto coupling at this

vasoconstrictor nerves of human omental blood vessels is clearly highly dependent on the frequency of nerve stimulation and on various local 'chemical' control mechanisms. One would expect that interference with this local control, and consequent increase or decrease in the amount of NA (number of NA 'quanta'?) secreted per nerve impulse would lead to considerable change in the effective NA concentration at the level of the receptors of the effector tissue and therefore to alteration in neuroeffector transmission. The consequent alteration in neurogenic tone in blood vessels would lead to cardiovascular complications unless the altered situation is coped with by a reflexly mediated change in the firing frequency of the vasomotor nerves. The magnitude of the reflex adjustment of firing frequency which would be required *in vivo* to compensate for the altered secretion of NA per nerve impulse during the highest degree of depression or facilitation of the secretory mechanism tested in the study is illustrated in Fig. 6. This estimation disregards the fact that reuptake of transmitter was blocked in the present work. The plot shows that the firing frequency in the nerves during depression of NA secretion by PGE_2 would have to be increased five-fold, from 1 Hz to 5 Hz, to maintain the NA output per unit time under facilitation by ETA/isoprenaline/phentolamine.

One would therefore conclude that even though cardiovascular symptoms need not necessarily be apparent as a result of disturbance of mechanisms for local feedback control of NA secretion, since reflex changes in firing frequency could easily compensate alteration in the amount of NA secreted per shock, at least during resting conditions, it is quite possible that such symptoms may become manifest in situations where the cardiovascular system is exposed to increased strain.

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Acta physiol. scand. 1977 101 211–218

From the Institute of Physiology University of Lund, Sweden

Effects of Sympathetic Nerve Stimulation in the Presence of a Slow Parasympathetic Secretion in the Parotid and Submaxillary Glands of the Rabbit

By

PER GJÖRSTRUP

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Abstract

[illegible] P *Effects of sympathetic nerve stimulation in the presence of a slow parasympathetic secretion in the parotid and submaxillary glands of the rabbit* Acta physiol. scand. [illegible]977 *101* 211–218.

[illegible] secretory responses from the parotid and the submaxillary glands of the rabbit were studied [illegible] excitation occurred either alone or during [illegible] slow parasympathetic background secretion, [illegible] the resting secretion normally present. The fairly rapid sympathetically evoked flow of saliva [illegible] parotid gland [illegible], as the background secretion, further increased and obtained at low frequencies, [illegible] sympathetic stimulation alone were subthreshold. The effects, [illegible] could be repeated [illegible] [illegible] drugs [illegible] pilocarpine induced secretion, were abolished by α- and β-adrenoceptor [illegible] in combination. The submaxillary gland gave [illegible] very scanty secretion on sympathetic excitation, [illegible] being mediated via β-adrenoceptors. The responses were not increased in [illegible] background secretion [illegible] effect of sympathetic activation [illegible] to retard the salivary flow. The retardation was attributed [illegible] accompanying sympathetic excitation. Eliminating the vasoconstrictor responses, [illegible] the sympathetic secretory effects, did not reveal any increase of the sympathetic salivary [illegible] in the submaxillary gland.

[illegible]ary secretion is generally considered to be controlled not only by parasympathetic, [illegible] also by sympathetic nerves. There are striking differences, however, between the secretory [illegible] to electrical excitation of the two types of nerves. Whereas the parasympathetic [illegible] regularly cause [illegible] copious flow of saliva, which can be maintained over long time [illegible], the sympathetically evoked secretion is usually fairly slow even at high stimulation [illegible] and tends to decline while stimulation is going on. The magnitude of the re[illegible] varies between glands of different species and between different glands in the same [illegible] there are even glands in which no salivary flow is obtained on sympathetic excitation. [illegible] dog, for instance, no saliva is secreted from the parotid, but some from the submaxillary [illegible], although the acini of both glands are surrounded by adrenergic fibres (see Schneyer [illegible] Emmelin 1974).

In recent expts. on dogs and cats the sympathetic nerve was found to have a p secretory effect when stimulated during a slow parasympathetically evoked secreti ing the resting secretion normally present in the waking state (Emmelin and Gjör 1976) The effect in the dog was particularly marked when sympathetic vasoconst prevented. Under these conditions a sympathetic secretory effect could be demon in the parotid gland of this species.

In rabbits histochemical observations show that the acini both of submaxillary glands are well supplied with adrenergic fibres (Ehinger Garrett and Ohlin 19 and Engel 1970 Kagajama and Nishijama 1972, Garrett 1974 Bloom, Carlsöö a son 1976) Physiological expts., on the other hand, indicate that sympathetic fi clear secretory effect in the parotid but very little or none at all in the submax of this species (Nordenfelt and Ohlin 1957 Morley Schachter and Smaje 1966, 1974 Smaje 1973 1974) Nordenfelt and Ohlin (1957) also noticed that a slow pa tion caused by pilocarpine could be accelerated by sympathetic stimulation whe secretion was retarded, probably because of vasoconstriction.

In the present expts the effects of sympathetic stimulation superimposed on a sympathetic background secretion were studied in the parotid and submaxillary g rabbit

Methods

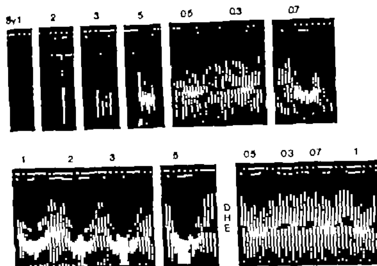
Rabbits of d fferent stra ns, 3–4.2 kg, 39 f r the s bmaxillary and 14 fo the parotid glan Urethane, 1.5 g/kg, was i itially gi en through a lateral ear vein and femoral ein was can dditional doses of the anaesthetic and fo injection of other drugs. A tracheal cannula cannulate the submaxillary duct the conjoi g tendon of the d gastric muscle as divided gently freed from the surround ng tissues, from the distal aspect of the mylohyoid muscle dow hil m of the gland The preparation of the duct lso made t possible t stimulate the chorda t n its course along the duct. The parotid duct was exposed and ca n lated as close to the mou and the auriculo-temporal nerve was exposed as described by Burgen (1964) fo cats and th pathetic trunk was dissected the neck F measurement of blood flow through the subm all veins emptyi g i to the external jugula ein except the mai gland lar one ere ligate tween ligatures. Heparin 1 000 I U./kg was given A polythene tube of 1 7 mm d and was inserted i to the jugular vein, the other nd bei g ttached t a ph toelectric drop reco

Stimulation A slow co ti u us flow f sali a, 1 3 drops/min, was produced by stimulati tympani at frequency of 0 5–3 Hz nd the riculo-temporal t 0.7 2 Hz, usi g d ratio supramaximal voltage. The sympathetic w s timulated t frequencies f om 0.07 to 10 Hz wit tion and supramaximal oltage. Bipolar electrodes f tted t Grass stim lators, S4 and S44 stimulus isolation units were used When pilocarpine was used t get slow continuous flow drug was infused by t bing p mp (Sage nstrument model 375A) t a rate of 3 μg/kg m

Recordings. In the submaxillary gland the spo taneous secretion nd the flow of saliv timulati g the sympathetic alone were measured by connecti g the cannula t horizontal 0 I the other experiments glass can la givi g drops of size of 15 μl were used. Drop rec made o the smoked drum. Drops of sal va were registered either by a ordinat writer manu or by an electromagnetic pen. Blood flow drops of blood w recorded by ordinat r by drop-recorder The blood pressure wa measured by connecting the left femoral artery manometer

Drugs Pilocarpine, adrenaline phenylephrine, isoprenaline dihydroergotamine, phenox practolol were given i.

Statistics Student's t-test f r pa red observ tions was used P-v lues less tha 0 05 were significant



Secretion from parotid gland. Records from above: minute marks, signal and secretion. In the first ... sympathetic (Sy; frequencies in Hz) ... stimulated alone and in the other sections during slow parasympathetic secretion. The background secretion ... evoked by stimulating the ... nerve ... in the upper sections at 1.1 Hz, in the first 2 lower sections at 1.5 Hz and in the last at ... Before the last section dihydroergotamine (DHE), 1 mg/kg, was injected.

Results

...d glands

...ne of the 14 parotid glands studied was there any sign of a spontaneous secretion. ...5 expts. the secretory responses to sympathetic stimulation alone were compared to ... obtained when excitation occurred during a parasympathetic background secretion ... seen in Fig. 1, the sympathetic stimulated alone at 1 Hz did not give any secre... 0.3 Hz was sufficient to accelerate the background secretion. The threshold frequency ...mpathetic stimulation was lowered from ...20 0.10 to 0.15 0.07 Hz (mean ± S.E.), ...erence which is highly significant (P 0.001). Further the latency for the onset of ...athetic secretion was considerably shortened. The amount of saliva produced on ...athetic excitation was increased when stimulation was superimposed on the slow para...athetic secretion: the average secretory responses were increased at 3 Hz from 5 µl/min ... µl/min. Maximal secretory responses were obtained around 3 Hz; higher frequencies ... retarded the flow of saliva, probably because of the simultaneous activation of ...constrictor fibres in the gland.

...e effect of sympathetic excitation, adrenaline or phenylephrine injections were also ...d against slow secretion set up by infusion of pilocarpine (Fig. 2). The responses to ...athetic stimulation were very similar to those seen in parasympathetic secretion: the ...ground secretion lowered the threshold from 1.83 0.31 Hz to 0.13 ± 0.05 Hz, n = 6 ... 0.01). Adrenaline also accelerated the pilocarpine elicited secretion and the threshold ... adrenaline evoked secretion was lowered from 5.20 1.23 µg/kg to 0.07 ± 0.03 µg/kg, ...5 (P 0.02). Maximal responses were obtained with doses around 5 µg/kg, while alone

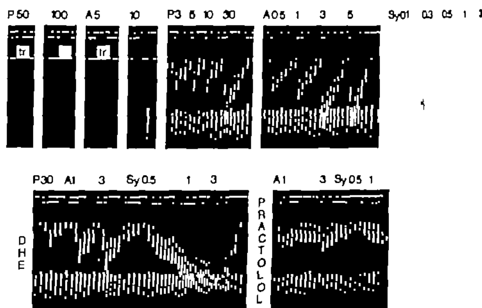


Fig 2 Secretio from parotid gland. Record as in Fig. 1 tr traces of secretion Secretion in th sections caused by drugs alone and in the others by drugs and sympathetic stimulation during pilocarpine in a dose of 3 / g/kg/min P phe ylephrine, A adrenaline. Doses in µg/kg are given first lower sectio d hydroergotami e (DHE), 1 mg/kg, was given and before the last section mg/kg

adrenaline in a dosage of 3 to 10 µg/kg in the different experiments only gave trace of saliva In 3 expts the α-adrenoceptor agonist phenylephrine in doses between 5 µg/kg bar ly evoked secretion, but in a pilocarpine secretion 1 to 3 µg/kg sufficed t the flow of saliva and maximal acceleration was obtained with 20 to 30 µg/kg.

Blocking the α-adrenoceptors with dihydroergotamine 1–2 mg/kg, reduced th ating effects of sympathetic stimulation and of adrenaline (Fig. 1 ?), while the re phenylephrine were almost or completely abolished (Fig. 2). The remaining ac effects of superimposed sympathetic stimulation or of adrenaline administrat however still produced at frequencies of stimulation or doses which acting a

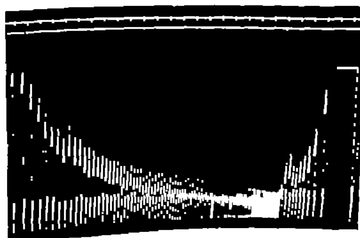


Fig. 3 Secretion fr gland. Records as Background secretion infusio of pilocarpin min. Th s secretion continuously increased nimal ceased breat cated by the signal f detail see t

eshold for secretion. These responses were apparently not due to an incomplete block but to an activation of β-adrenoceptors, since they were greatly reduced shed by the β_1-adrenoceptor blocking agent practolol, 2–4 mg/kg (Fig. 2) I two 4 animals this agent in a dose of 2 mg/kg was seen to have small transient accelerating on the background secretion. An intrinsic secretory property of this drug has also been ed in the rat (Ekstrom 1969).

3 shows a slow pilocarpine induced secretion that suddenly was accelerated and ed to be so for a period of 20 min until the animal gave up breathing. At the same he pupils were widely dilated though the sympathetic trunk had been cut on the right nd 0.5 mg/kg of dihydroergotamine given 2 h earlier The resemblance between this ation and those on superimposed sympathetic stimulation or injections of adrenaline it likely that the increase in secretion was due to the release of catecholamines from renals when the animal was in its final state. This idea is supported by the observed tion of the pupil, which further indicates that the catecholamines were acting not on β- but also on α-adrenoceptors in this case.

...ndibular glands

n the cannula was connected to the horizontal pipette a slow continuous flow of saliva, n to be spontaneous, was seen (Nordenfelt and Ohlin 1957 Smaje 1973 1974) It was l to be 0.25 µl/min on the average (n 7) and varying between 0 1 and 0.4 µl/ml in rent animals when studied in 15 min periodes.

...mulation of the sympathetic trunk produced i most rabbits an additional 0.1–0.5 µl/ of saliva at stimulation frequencies between 0.5 and 3 Hz; this additional secretion was st doubled at 5 Hz. The threshold frequency for secretion was in 1 animal found to be Hz, in 5 animals 1 Hz, in 2 animals 2 Hz and in one 3 Hz. In 3 animals no secretion was ...ned on sympathetic excitation. Occasionally at 5 Hz and generally at higher frequencies low of saliva seen at the onset of stimulation successively declined during the stimulation ...d When this was the picture the spontaneous secretion was slowed or even bsent ...ng the first minutes after stimulation. After administration of dihydroergotamine, 1 3 ...kg, the tendency to decline was less marked and a more continuous flow of saliva was ...ned the change after blockade was probably due to a reduced vasoconstriction. Block the α-adrenoceptors did not, however affect the amounts of saliva produced at fre ...ncies between 0 5 and 3 Hz.

he saliv on sympathetic stimulation, whether secreted before or after α-adrenoceptor ...kade, was always viscous. This necessitated nterposed chorda stimulations to wash the sympathetic saliva otherwise causing obstruction. Further also seen in the expts, ...re stimulation of the sympathetic did not give any increased flow the first drops on the ...owing chorda stimulation were regularly more viscous than usual.

The effects of sympathetic stimulation when superimposed on a slow parasympathetically ...uced flow ere as follow In 8 rabbits no change in salivary flow was obtained, in 4 ...mals there was a small increase in salivary secretion, but judging from the experiments ...cribed above not greater than a mere addition of sympathetically evoked saliva In a ...rd group of 8 animals excitation of the sympathetic slowed the salivary flow rate. The

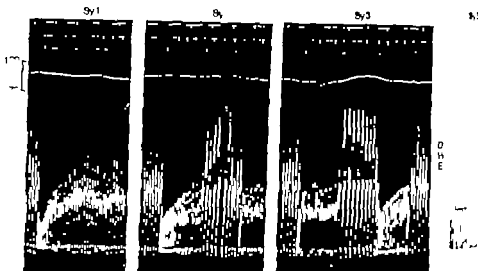


Fig. 4 Secretion and blood flow from submandibular gland Records from above minute mark signal, blood pressure and blood flow Bet een signal mark the sympathetic as stimulated du parasympathetic secretion, indicated by l wering the signal l ne. The chorda tympani as at Hz. Befor the last section dihydroergotamine (DHE) mg/kg i. w given.

retardation of the secretion was regularly accompanied by a retardation in blood flo the gland (Fig. 4) To overcome the vasoconstriction, the most probable cause f creased salivary flow the α-adrenoceptor blocking drugs dihydroergotamine, 1 or phenoxybenzamine 5–10 mg/kg, were given After α-adrenoceptor block stimulation frequencies were needed to retard the parasympathetic background and to reduce the blood flow through the gland. On lower frequencies the α-adr blockade was not seen to have any marked effect on the secretory responses the b secretion was either unchanged, or only slightly increased although the vasoconstri eliminated (Fig. 4) In 8 rabbits injection of the β-adrenoceptor stimulating no during a slow parasympathetic secretion regularly increased the salivary flow with the dose given 0 1–5 μg/kg, an additional 2–10 μl of saliva was formed o period of 2–4 minutes. When tested on its own isoprenaline, 5 μg/kg gave a se about 5 μl, except for one animal in which no secretion was seen. Secretory respon same magnitude to isoprenaline have earlier been reported by Ohlin (1964)

Discussion

In agreement with earlier reports the present expts. show that sympathetic stimulat causes a fairly rapid secretion from the parotid, but a minute response only or s none at all from the submaxillary gland of the rabbit. The parotid secretion wa mediated via α-adrenoceptors but not exclusively as pointed out by Nordenfelt a (1957). Since the effect remaining after -adrenoceptor block could be abolished by i it can be concluded that it was evoked via receptors of the β-subgroup Adrenoc the β-type mediating salivary secretion in other species also belong to this subgrou 1972, Gjörstrup 1975).

The sympathetic secretory effect was greatly augmented in the parotid gland whe

presence of slow parasympathetic resting secretion, an experimental situation con- to be more physiological than stimulating separately the sympathetic nerves (see and Emmelin 1961). The same was true when sympathetic nerve stimulation was d by sympathomimetic drugs. In these respects the gland reacts as salivary glands of d dogs (Emmelin and Gjörstrup 1975, 1976). In the dog submaxillary gland stimula- the sympathetic during a slow parasympathetic secretion produces an initial very low of saliva, followed by a phase of accelerated flow at a fairly well maintained level. t phase is attributed to myoepithelial contraction and the second to activity in se cells (Emmelin and Gjörstrup 1974). However the present increase in salivary flow he parotid gland occurred gradually and evenly which makes it unlikely that there initial acceleration due to myoepithelial activity.

ontrast, no augmentation could be detected in the submaxillary gland of the rabbit. gland the main effect of sympathetic stimulation was to reduce the background and spontaneous secretion and in view of the pronounced vasoconstrictor effect ac- nying the stimulation, it is reasonable to attribute the retardation of the salivary flow minished blood supply. Small secretory responses can be elicited by isoprenaline in bmaxillary gland of the rabbit, as also shown by Ohlin (1964). From this observation be inferred that the glandular cells have a sparse supply with β-adrenoceptors and that onal sympathetic fibres may have access to some of these in most rabbits. The small ory effect of sympathetic stimulation is considered to be evoked via β-adrenoceptors e 1973, 1974). Here, it is possible to study secretion when an α-blocking substance has iven to abolish vasoconstriction. Not even then could augmentation of the sympathetic nse be produced by supplying a background secretion.

the parotid gland of the dog adrenergic fibres are present in the acini (Fujiwara *et al* Garrett and Holmberg 1972), and a sympathetic secretory response, lacking when the is excited alone, can be revealed by adding the background activity and abolishing ympathetic vasoconstriction (Emmelin and Gjörstrup 1976). The present analogous ments do not support the view that sympathetic fibres greatly contribute to the trans- of water to the acini of the submaxillary gland of the rabbit. Recent work has shown ubmaxillary gland to receive less intimate and a less plentiful autonomic innervation, sympathetic and parasympathetic, than the parotid (Garrett, personal communication) may partly account for the difference between the glands as regards secretion of water sponse to sympathetic nerve stimulation. However it cannot be the full explanation; as seen in methods, the secretory responses to parasympathetic stimulation are similar oth glands.

is reasonable to attribute other functions to the adrenergic nerves surrounding the acini e submaxillary gland. They may be of importance for the secretion of proteins; this is orted by the present finding that the submaxillary saliva became very mucous when the pathetic nerves were activated. Langley already in 1879 pointed out that both the parotid the submaxillary glands became degranulated on sympathetic excitation. Although not ported by the present experiments, it is also conceivable that the sympathetic acinar nerves part in the control of the myoepithelial cells surrounding the acini, as is the case in dogs.

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Acta physiol. scand. 1977. 101. 219-229

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Fever and Behavioural Temperature Regulation in the Frog *Rana esculenta*

By

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Abstract

...RE, K., M. CABANAC and G. MYHRE. *Fever and behavioural temperature regulation in the frog Rana esculenta.* Acta physiol. scand. 1977. *101.* 219–229.

...in and colonic temperatures were recorded in frogs (*Rana esculenta*) which had selected suitable environment in a box filled with 2–3 cm water. The water temperatures ranged from 0°C to +40 C. ...measurements were performed before and after intraperitoneal injections of killed pathogenic bacteria ...and *M. ranae*), killed non-pathogenic bacteria (*M. aquae II*) and 0.9 sterile saline, intra-...neal injections of blood plasma from frogs pre-injected with killed *M. ranae*, injections of PGE_1 int... The injections of pathogenic bacterial endotoxin caused, after latencies of 5–120 min, higher pre-...d water temperatures, which produced an average maximum colonic temperature increase of 6.5°C+ ...(S.E.) (p 0.001). The non-pathogenic bacteria and sterile saline caused no temperature change. ...phasic hyperthermia of shorter latency was caused by injections of blood plasma from frogs pr...ted with *M. ranae*. Monophasic hyperthermia of the shortest latency was observed after diencephalic ...tions of PGE_1. Based on their similarity we suggest that ectothermic and endothermic fever hav... ...mon phylogenetic origin.

...words: Frog, behaviour, temperature regulation, fever, prostaglandins

...ver is a pathological condition with raised core temperature maintained by activation of thermoregulatory mechanisms of the organism. This manifestation of disease is charac...istic for most mammals during inflammations and has been extensively studied and ...iewed (Bligh 1973, Hensel 1973, Hellon 1974).

Both bacteria and viruses produce fever by injection into mammals (Hellon 1974). This ...er is characterized by a typical pattern of temperature changes including a dose–response ...lationship concerning maximum temperature increase, latency and duration of the fever. It ...generally accepted that the exogenous pyrogens induce release of endogenous pyrogens ...the organism (Bligh 1973, Hellon 1974). Further several authors (Cooper, Cranston and ...nour 1967, Milton and Wendlandt 1970, Feldberg and Gupta 1973, Stitt 1973, Cooper

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Preston and Veale 1976) have produced evidence suggesting that the central action of endogenous pyrogens consists of initiating synthesis of Prostaglandin E (PGE_1) which modifies the thermoregulatory neuronal activity in the CNS

Recently Vaughn Bernheim and Kluger (1974) have reported that when the lizard (*Dipsosaurus dorsalis*) was injected with killed Gram-negative bacteria (*Aeromonas h*...) it selected higher ambient temperatures and increased its cloacal temperature by approximately 2 C Reynolds, Casterlin and Covert (1976) injected endotoxin from the same bacteria into two species of fish (*Lepomis macrochirus Rafinesque* and *Micropterus sal*...) and observed that they increased their average preferred water temperature by 2.6 C ... when killed *Aeromonas hydrophila* was injected into tadpoles of *Rana catesbeiana* and *R. pipiens* they selected 2 6 C and 2.7 C higher water temperatures (Casterlin and Reynolds 1977)

The role of behaviour in producing fever in neonatal endotherms was demonstrated by Satinoff McEwen Jr and Williams (1976) Newborn rabbits maintained under ... thermal conditions did not develop fever upon injection of exogenous pyrogen, but when placed in a thermal gradient they increased their rectal temperature with 0.9 C by selecting a warmer location

The role of mammalian fever in decreasing the mortality during bacterial infection is disputed (Bennett and Nicastri 1960) However in the lizard *Dipsosaurus dorsalis*, the survival value of the behavioural fever response has been indicated (Kluger Ringler and Anver ... Bernheim and Kluger 1976 a) After receiving injections of live *Aeromonas hydrophila* ... were 1 allowed to increase their temperature behaviourally 2, maintained at their ... temperature 3 injected with sodium salicylate which depressed the fever response The highest per cent of survival was observed in the first group Comparative studies of ... thermic and endothermic fever might shed light on the concept of a common phylogenetic origin and thus the role of fever as a possible defense mechanism.

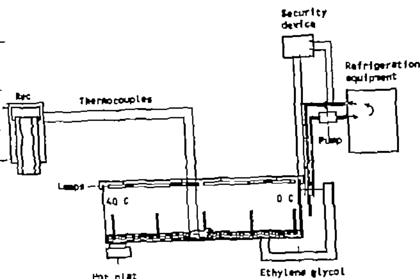
The frog *Rana esculenta* selects a location in a thermal water gradient which produces colonic temperatures between 25 C and 28 C (Cabanac and Jeddi 1971). This temperature regulating behaviour is triggered by signals from spinal thermoreceptors (Duclaux, ... and Cabanac 1973) It thus represents a useful preparation for studying the characteristics of behavioural fever after injections of pyrogen

In this study the thermoregulatory behaviour of the frogs was studied after intraperitoneal injections of killed pathogenic and nonpathogenic bacteria Further another group of frogs received intraperitoneal injections of blood plasma from frogs pre-injected with pathogenic bacterial endotoxin, while a third group received injections of PGE into the brain.

Materials and Methods

Animals. Frogs (*Rana esculenta*) weighing from 15 g to 145 g were bought at the local market and ... without food at room temperature. Only healthy frogs, as judged by their behaviour were used

Temperature gradient The temperature gradient was established in a box made of galvanized ... cm long, 15 cm wide, 37 cm deep (Fig. 1), filled with 3 cm of water The water temperature in one end of the box was maintained at 0 C by circulating cooled ethylene glycol ... surrounding insulated compartment An adjustable heater was located underneath the other end of the box and maintained the water temperature in this end at about 40 C ...



Schematic drawing of the experimental design (modified from Duclaux, Fantino and Cabanac

[illegible] reduce possible disturbance of the frog from the surroundings the gradient [illegible]as uniformly illu[illegible]ted by overhead lamps and partly covered by [illegible] lid Observation of the animal [illegible]as accomplished by [illegible] of mirrors

[illegible] measurements Temperatures were measured by 36-gauge nylon coated copper-constantan [illegible]couples [illegible]ith [illegible]he reference junctions in melting ice, and registered on [illegible] Polycomp 2, 12 channel [illegible] recorder (Hartmann & Braun A G) The accuracy of the measurements [illegible]as 0 3°C

[illegible] temperature [illegible] which [illegible]as preferred by [illegible]he animal [illegible]as obtained by suturing [illegible] thermocouple to [illegible] on the abdomen

[illegible] colonic temperature [illegible]as measured by [illegible] thermocouple 3 cm into [illegible]he colon, fastened by external [illegible]

[illegible] thermocouple leads did not impair the frogs movement in the gradient

[illegible] procedure The general protocol was [illegible] allow the frogs to select [illegible] preferred temperature [illegible] thermal gradient before and after injections of pyrogens Group 1 received intraperitoneal injections [illegible] endotoxin, group 2 blood plasma from frogs pre-injected [illegible] bacterial endotoxin, [illegible] group [illegible] injections of Prostaglandin E_1 into the brain Skin and colonic temperatures [illegible]ere continuously [illegible] during the [illegible]hole expt

[illegible] expts were initiated by equipping the frogs [illegible]ith the thermocouples In order to diminish the animal [illegible] skin anesthesia [illegible]as produced by cooling the frog in ice. The animal [illegible]as then put into the gradient [illegible] temperature, and left undisturbed until it had selected [illegible] preferred [illegible] temperature [illegible] at least 30 min in the same location) The frog [illegible] then carefully removed from the gradient and [illegible]ection [illegible] administered Following the injection the frog [illegible]as replaced in the gradient at its preferred [illegible], and left undisturbed throughout the expt Care [illegible]as taken to avoid noise and any disturbance of [illegible] animal

[illegible] pyrogen Ten frogs were injected [illegible]ith bacterial pyrogen. Three strains of Mycobacterium [illegible] were used The bacteria were killed by heat ([illegible] 80 C), suspended in [illegible] 0 9 [illegible] saline (1 mg/ml) and stored in the refrigerator ([illegible] 4 C) The frog received 2 ml of this solution [illegible] Each frog also received an i p control injection of [illegible] ml sterile 0 9 [illegible] saline The control expts, and [illegible] expts, were performed at the same time of the day

[illegible], sterile needles and syringes were used for all injections The solutions of killed Mycobacterium [illegible] transferred to the syringe close to the flame from [illegible] Bunsen burner in order to avoid contamination [illegible] solutions by foreign bacteria Before injection all solutions were brought to room temperature.

Preston and Veale 1976) have produced evidence suggesting that the central action of endogenous pyrogens consists of initiating synthesis of Prostaglandin E_1 (PGE$_1$) which modifies the thermoregulatory neuronal activity in the CNS

Recently Vaughn, Bernheim and Kluger (1974) have reported that when the lizard (*Dipsosaurus dorsalis*) was injected with killed Gram-negative bacteria (*Aeromonas hydrophila*) it selected higher ambient temperatures and increased its cloacal temperature by approximately 2 C Reynolds, Casterlin and Covert (1976) injected endotoxin from the same bacteria into two species of fish (*Lepomis macrochirus Rafinesque* and *Micropterus salmoides*) and observed that they increased their average preferred water temperature by 2.6 C. F... when killed *Aeromonas hydrophila* was injected into tadpoles of *Rana catesbeiana* and *R. pipiens* they selected 2.6 C and 2.7 C higher water temperatures (Casterlin and Reynolds 1977)

The role of behaviour in producing fever in neonatal endotherms was demonstrated by Satinoff McEwen Jr and Williams (1976) Newborn rabbits maintained under constant thermal conditions did not develop fever upon injection of exogenous pyrogen, but placed in a thermal gradient they increased their rectal temperature with 0 9 C by selecting a warmer location

The role of mammalian fever in decreasing the mortality during bacterial infection is disputed (Bennett and Nicastri 1960) However in the lizard *Dipsosaurus dorsalis*, the survival value of the behavioural fever response has been indicated (Kluger Ringler and Anver ... Bernheim and Kluger 1976 a) After receiving injections of live *Aeromonas hydrophila* ... were 1 allowed to increase their temperature behaviourally 2, maintained at their ... temperature 3 injected with sodium salicylate which depressed the fever response ... highest per cent of survival was observed in the first group Comparative studies of ecto-thermic and endothermic fever might shed light on the concept of a common phylogenetic origin and thus the role of fever as a possible defense mechanism

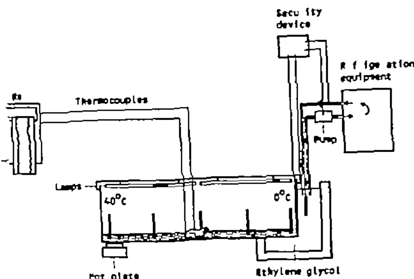
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In this study the thermoregulatory behaviour of the frogs was studied after intraperitoneal injections of killed pathogenic and nonpathogenic bacteria Further another group of ... received intraperitoneal injections of blood plasma from frogs pre-injected with pathogenic bacterial endotoxin while a third group received injections of PGE into the brain.

Materials and Methods

Animal Frogs (*Rana esculenta*) weighing from 15 g to 125 g were bought at the local market and ... without food at room temperature. Only healthy frogs, as judged by their behaviour were used

Temperature gradient The temperature gradient was established in ... box made of galvanized ... cm long, 15 cm wide, 37 cm deep (Fig. 1), filled with 3 cm of water The water temperature at one end of the box was maintained at 0 C by circulating cooled ethylene glycol ... a surrounding insulated compartment An adjustable heater was located underneath the other end of the box and maintained the temperature in this end at about 40 C F... thermometers were spaced at regular intervals ...



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Disposable, sterile needles and syringes were used for all injections The solutions of killed Mycobacterium transferred to the syringe close to the flame from Bunsen burner in order to void contamination he solutions by foreign bacteria Before injection all solutions were brought to room temperature.

F f

Transfer f endogenous pyrogen Killed *M ranae* was i.p. injected into 5 donor frogs in doses r from 53 / g/g to 75 / g/g. About 2 h later the blood was drawn and the frog killed.

The blood sampling procedure consisted of carefully dissecting the heart free and inserting a fire in the ventricle After injecting a small amount of heparin the blood was drawn into a syringe and c f ged for 10 min at 3 000 r p.m.

0 5 to 2.5 ml of this plasma was i p. injected into 4 recipient frogs according to the general pro. the expts.

Prostaglandin E The prostaglandin was supplied as PGE_1. It was made soluble in 0 9 saline verting it to PGE Na as described by Stitt (1973) Stock solutions of 10 mg PGE_1–Na/ml and 1 mg Na/ml were made. All glassware used in this connection was scrupulously cleaned and dried

5 frogs received PGE i jections. After they were removed from their initial stay in the gradient they *were locally cooled by ice cubes. The skull was exposed and hole carefully made with a dental* slightly left of the midline, in an area correspondi g to the diencephalon.

The injections into the brain were administered by a Hamilton syringe fastened in a micromanip. The syri ge needle was carefully lowered down through the hole until its free progress was arrested by bony structures of the palate. The needle was then retracted 1 mm and the solution injected.

In every expt 2.5 / l of PGE Na solution was injected. The control injections were done later the *day and consisted of 2.5 μl f the sal ne vehicle, administered through the same hole in the skull*

When the expt. was terminated, 2.5 / l of 5% methylene blue was i jected according to the &c procedure, and the animal killed by decapitation. The brain was dissected free, fixed in a 10 % form solutio and macroscopically inspected to verify the site of the injection.

T eatment of the results

The t tistical significance of the observed response was assessed with Student s *t*-test.

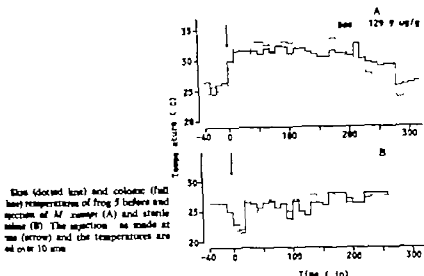
Results

Bacterial pyrogen

A *Typical response* Fig. 2 shows the results from 2 expts. on one frog (*5*), which is r sentative for the study The animal settled down with a skin temperature of about and a colonic temperature of 26 C After receiving an i p injection of *Mycobacterium xenopi* (panel A) the frog moved to warmer water and during 220 min the skin perature varied between 30 8°C and 33 3 C while its colonic temperature was main between 30 5 C and 32.5 C After this period of fever the frog returned to cooler wa indicated by the rapidly falling skin temperature, followed by a slower drop of the co temperature When the frog, on the previous day received an i p. injection of sterile saline (Fig. 2, panel B), no major change of location occurred except for an initial *lasting "cool trip"* The frog s colonic temperature was most of the time maintained be 4 C and 27°C, increasing to 28°C in the last hour

As a general trend, illustrated in Fig. 2, the skin temperature remained higher tha colonic temperature during the fever period but lower than the colonic temperature the frog returned to its pre-injection thermal condition In the control expts. the skin perature oscillated around the colonic temperature Further when the frog was rem from its selected location in the gradient, it always returned quickly This observation p valid whether the animal was hyperthermic or normothermic.

B *Average response* Fig. 3 panel A shows the mean colonic temperatures of 7 frogs 3 4 5 7 and 8) before and after the injection of *Mycobacterium xenopi* in doses ra from 16 1 μg/g to 129 9 μg/g. The average response of the 7 frogs is not calculated be 150 min after the injection since the first frog terminated its fever at this



...tion (dotted line) and colonic (full line) temperatures of frog 5 before and ...njection of *M. ranae* (A) and sterile saline (B). The injection ...as made at ...me (arrow) and the temperatures are ...ed over 10 min.

...d that, following the injection, the frogs increased their colonic temperatures, *Le* ...e feverish.

...the control expts., shown in the same panel, all of the frogs except one were injected ...sterile 0.9% saline (frog 2 died after being accidentally affected by the needle in the ...l cord). The control injections produced no hyperthermia. A slight elevation of the ...ic temperatures towards the end of the expts. was, however, noted (Fig. 3 A and ...2 B). T... investigate if this observation was due to ... diurnal temperature pattern, all ...olonic temperatures obtained in the control expts. were related to the time of the day ...a regression line was fitted according to the method of the least squares. Although this ...indicated a 1°C higher colonic temperature at 5 o clock p.m. compared to at 11 ... clock ... this trend was statistically non-significant (p ... 0.5).

...o frogs (10, 11) were injected with *Mycobacterium ranae* in doses of 47.6 µg/g (10) and ... µg/g (11). Within 40 min frog 11 had moved to warmer water and increased its colonic ...erature from 22.8°C to 31.9°C. It then died during the hyperthermia, 60 min after the ...tion. Examination of the body did not reveal any signs of internal damage caused by the ...le. Frog 10 exhibited ... latency of 20 min and then entered a hyperthermic period lasting ... min. The fever described a clear biphasic pattern. The control injection of sterile 0.9 ... ...e produced no hyperthermic response (Fig. 3 B).

...e maximum colonic temperature increase, maintained for 30 min, ...veraged from the ...ogs which received injections of *M. xenopi* and *M. ranae* was 6.5°C ± 1.0°C (S.E.M.) ... 0.001), while the corresponding temperature change at the same time after ...njecting ...le saline was non-significant (p ... 0.3).

... Non-pathogenic bacterium. When 2 frogs (5, 9) were injected with *Mycobacterium* ...e *II* in doses of 139.9 µg/g and 56.3 µg/g respectively no hyperthermic response was ...ed (Fig. 3 C).

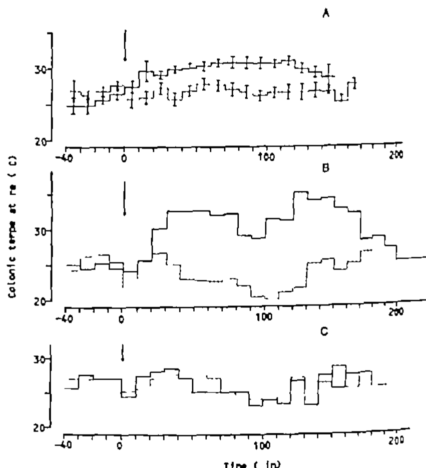


Fig 3 Panel A show the erage colonic temperat res and standard error of the mea (vertical before and after I ject on f killed *M xenopi* concentrations ranging from 16 1 µg g 1 1?4 µg 1 drawn l ne) n frogs 1 2, 3 4 5 7 d 8, nd sterile 0 9 saline (dotted l ne) n frogs 1 3 4, 5, 7 Panel B exhibits colonic temperatures n one frog (10) I jected with 47 6 µg g k lled *M* (full l ne), and terile 0 9 saline (dotted line). Panel C show the colonic temperatures in tw frogs (1 139 9 µg g, full draw l ne 9 dose 56 3 µg g, dotted line) jected w th killed *M* Further as fig —

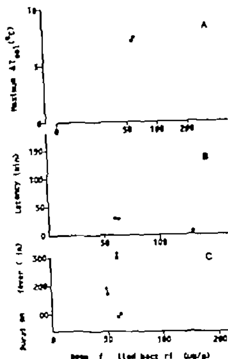
D Characteristics of the response The maximum colonic temperature increase tained for 30 min, during fever in each frog showed a tendency to increase up to the 60 µg/g, while at the highest dose (130 µg/g) a lowered response was observed (Fig

Latency is in this study defined as the time elapsed between the injection and the of colonic temperature increase As illustrated in Fig. 4 B latencies of 120 min and 5 min observed at the lowest and highest dose of bacteria respectively while those of the doses ranged from 10 min to 40 min

Two frogs were still hyperthermic when the expts were terminated after fever dur of 170 min and 300 min The other frogs returned to cooler water exhibiting fever dur ranging from 90 min to 240 min (Fig. 4 C)

Transfer of endogenous pyrogen

Table I shows the characteristics of the response observed in four frogs (20 injected with blood plasma from frogs pre-injected with *M* ter a la



Panel A shows the maximum increase of the temperature which was maintained for 30 min to the logarithm of the administered dose of bacteria. In panel B and C the latency and duration of the hyperthermic response are related to dose of killed bacteria given. Closed circles, ... *M.* ... open circles injections of *M.* The small arrows in panel C indicate that the ... were still hyperthermic when the expts. were ...

...ogs moved towards warmer water and displayed a monophasic hyperthermia of mod... size, lasting 16–20 min. The mean increase of the colonic temperatures ranged from ... to 5.3°C. Two of the frogs died respectively 30 min and 3 h after the injections, but the ... of the deaths could not be determined. When 3 frogs received control injections of 1.1 ... ml plasma from normal frogs, no change of colonic temperatures occured.

...staglandin E_1

...l of the Prostaglandin E solution was injected into the diencephalon of 5 frogs (9, 15, 16, 18) in doses ranging from 54 ng/g to 141 ng/g. The response (Table II) followed a general

...E I. Characteristics of the hyperthermic responses exhibited by the frogs receiving intraperitoneal injections of blood plasma from frogs pre-injected with killed *M.* ...

...	Injected Amount ml	Dose µl/g	Latency of response min	Duration of response min	Mean increase of T colonic °C
	2.3	65.6	4	20	3.9
	0.3	76.5	4	20	3.0
	0.5	18.3	4	16	2.0
	2.0	100.0	3	20	5.3

Dead 30 min after the injection.
...ad 3 h after the injection.

TABLE II Characteristics of the hyperthermic responses exhibited by the frogs receiving PGE₁ prostaglandin E_1 (PGE) into the brain. The bracketed numbers denote the colonic temp deviations from the pre-injection levels, obtained after the control injections at the time corresponding to the PGE_1 induced hyperthermia.

Frog	PGE		Latency of response min	Duration of response min	Mean increase of T colonic °C
	Amount µg	Dose ng/g			
9	5	141		65	5 8
15	2.5	69		35	8.8 (−0 8)
18	2.5	58	2	.5	9 7 (1 0)
16	.5	83		20ᵃ	6.5
17	2.5	54	3	28ᵃ	7.5

The animals died during the hyperthermic phase

pattern which is illustrated by one expt. (frog 15) in Fig. 5 After a latency of 2 min the moved towards warmer water thereby producing a monophasic hyperthermia lasting min. Control injection in the same brain area produced no hyperthermic response frogs (16, 17) developed signs of nervous disorder (impaired and jerky movements of the extremities) shortly after the injections They did, however move to warmer locations died there 22 min (frog 16) and 31 min (frogs 17) after the injection (Table II). A explanation could be that with these two particular frogs physical damage to the thalamus was caused by the syringe needle Since the ventral thalamus is a motoric ordinating centre (Romer 1970 p. 519) in the frog, the observed motoric dysfunction well result

Macroscopic examination of the brains showed that the frogs were injected in diencephalon slightly left of the midline and close to the optic chiasma

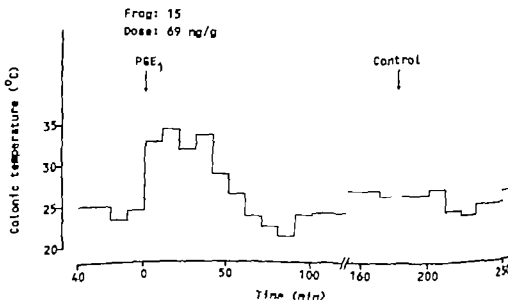


Fig 5 The colonic temperatures of one frog (15) before and after injection of .5 µg Prostaglandin (PGE) and sterile 0.9 saline (control) the diencephalon The Prostaglandin solution was injected at zero time and the saline 180 min later

Discussion

...gs in the present study voluntarily selected water temperatures which produced ... temperatures between 25°C and 28°C thus confirming the observations of Cabanac ...(1971) and Duclaux, Fantino and Cabanac (1973). When killed *M. xenopi* and ...e were injected i.p. in doses ranging from 16 µg/g to 130 µg/g, the frogs shifted their ...ns to warmer water which significantly increased their colonic temperatures ($p < 0.001$). *M. xenopi* is pathogenic towards anurans (Schwabacher 1959), *M. aquae II* is clearly ...thogenical and *M. ranae* is only suspected pathogenic (Carret 1973). The fever re... produced by the frogs which received endotoxins from *M. ranae* may lend support ...possible pathogenity of this bacterium, since no fever response was encountered when ...ae II was injected. These observations show that a behavioural fever was produced ...p. injections of endotoxins from pathogenic bacteria. Thus it is evident that in addition ...curring in reptiles after injection of live and dead *Aeromonas hydrophila* (Vaughn, ...im and Kluger 1974. Bernheim and Kluger 1976 a, b), fish (Reynolds, Casterlin and ...t 1976) and amphibian larvae (Casterlin and Reynolds 1977), behavioural fever occurs ...adult amphibian.

...lowing injections of exogenous pyrogen the hyperthermia occurred after delays from ... to 120 min. When recipient frogs were injected with blood plasma from frogs pre...ed with *M. ranae* all observed latencies were shorter. In accordance with Grant and ...s (1953) observations in rabbits. Although the present results are too few to rep... conclusive evidence, they may indicate that the blood of the donor frogs contained ...enic substances different from bacterial endotoxins since the latencies were shorter ...e shortest latency was observed when PGE was injected into the hypothalamic region ... frog's brain. This is in accordance with Stitt's (1973) observations in rabbits and indi... that PGE_1 in this brain region of the frog induce a hyperthermic response brought about ...lecting higher preferred temperature.

...e latency observed when exogenous pyrogen was i.p. injected could be due to delayed ...al absorption. However the consecutive shortening of the latencies in the three groups ...pts may also indicate that the exogenous pyrogen did not exhibit its effect directly on ... but acted through release of endogenous pyrogen which in its turn modified the ...al nervous thermoregulatory activity in connection with synthesis of PGE. This is a ...rally accepted view of the processes involved in generating fever in mammals (Hellon ...).

...e duration of the frog's fever response and the relationship between the colonic tem...ture increase and the dose of bacterial endotoxin were similar to corresponding observa...in rabbits (Eichenberger *et al.* 1966, Lechat et Deleau 1964), supporting the indicated ...larity between ectothermic and endothermic fever.

...e fever obtained in rabbits after injecting pyrogenic blood was similar to bacterial ...ced fever regarding magnitude and duration (Grant and Whalen 1953). When the frogs ... injected with blood plasma from donor frogs pre-injected with *M. ranae* the magnitude ...duration was modest compared to when exogenous pyrogen was injected. This may be ... to several factors. Observations of Grant and Whalen (1953) and Snell (1961) suggest low

concentrations of endogenous pyrogen in the blood of rabbit and man, and we obtained volumes of blood from the donor frogs. Finally according to Grant and Whalen (19 pyrogenity of the rabbit s blood was dependent upon the time elapsed after bacterial toxin was injected. Accordingly we may not have drawn the blood of the frog at the maximum pyrogenity

When PGE_1 was injected into the hypothalamus of the frog the magnitude of the colonic temperature increase was in the range observed when bacterial endotoxin injected This pattern is in accordance with results obtained in rabbits (Stitt 1973, Preston and Veale 1976) and represent a further support of the similarity in the generating ectothermic and endothermic fever

Finally the present study permits some conclusions about the organization of temperature regulation in amphibians. The existence of cutaneous temperature sensitivity is since the frogs orient themselves in a thermal gradient (Cabanac and Jeddi 1971 Fantino and Cabanac 1973) When the frogs in the present study were moved away from thermopreferendum they promptly returned before any change of the colonic temperature was evident This observation further supports the existence of skin thermoreceptors ternal signals from spinal temperature receptors have been demonstrated (Duclaux, and Cabanac 1973) and the fever induced by PGE_1 injections in the hypothalamus frog suggests that this brain region is involved in temperature regulation.

When the frogs in the present study had selected a preferred water temperature, the signals in their thermoregulatory system presumably equalled some reference point) The selection of a higher thermopreferendum by the frogs after injections of genic substances is most easily explained by a raised set point, which at pre-injection ferred water temperature will induce heat seeking activities. This observation is in connection with the challenge of the concept of set point in temperature regulation chell, Snellen and Atkins 1970 Houdas *et al* 1973)

d Alecy and Kluger (1975) and Pittman *et al* (1976) have produced evidence similarity between avian and mammalian fever Although endothermic fever is the increased metabolism and peripheral vasoconstriction in addition to behaviour while thermic fever is a sole behavioural response, the present results may indicate that responses are initiated through similar intermediary steps. Since the general pattern of system as it is known in mammals pre-exists in an amphibian, there seems to be reason propose that ectothermic fever precedes that in the endotherms Thus the present support the theory of a common phylogenetic origin of ectothermic and endothermic

The present work was supported by l'I stitut National de la Sa té t de la Recherche M (I.N.S.E.R.M.), le Centre National de la Recherche Scientifique (C.N R.S.), la Fondation pour la Rech Médicale Française and The Norwegian Research Council fo Science and the Humanities. We Professor J Vallier Raynard, Department of Bacteriology and Doctor J E. Pike, The Upjohn Co who provided free bacteria and prostaglandin respectively

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concentrations of endogenous pyrogen in the blood of rabbit and man, and we obtained volumes of blood from the donor frogs. Finally according to Grant and Whalen (19 pyrogenity of the rabbit s blood was dependent upon the time elapsed after bacterial toxin was injected. Accordingly we may not have drawn the blood of the frog at the maximum pyrogenity

When PGE_1 was injected into the hypothalamus of the frog the magnitude of the colonic temperature increase was in the range observed when bacterial endotoxin injected This pattern is in accordance with results obtained in rabbits (Stitt 1973, Ca Preston and Veale 1976) and represent a further support of the similarity in the pro generating ectothermic and endothermic fever

Finally the present study permits some conclusions about the organization of temperature regulation in amphibians. The existence of cutaneous temperature sensitivity is sug since the frogs orient themselves in a thermal gradient (Cabanac and Jeddi 1971 D Fantino and Cabanac 1973) When the frogs in the present study were moved away from thermopreferendum, they promptly returned before any change of the colonic temper was evident This observation further supports the existence of skin thermorecep ternal signals from spinal temperature receptors have been demonstrated (Duclaux, Fa and Cabanac 1973) and the fever induced by PGE injections in the hypothalamus of frog suggests that this brain region is involved in temperature regulation.

When the frogs in the present study had selected a preferred water temperature, the signals in their thermoregulatory system presumably equalled some reference level point) The selection of a higher thermopreferendum by the frogs after injections of p genic substances is most easily explained by a raised set point, which at pre-injection ferred water temperature will induce heat seeking activities. This observation is imp in connection with the challenge of the concept of set point in temperature regulation chell Snellen and Atkins 1970, Houdas *et al* 1973)

d'Alecy and Kluger (1975) and Pittman *et al* (1976) have produced evidence sugge similarity between avian and mammalian fever Although endothermic fever is the re increased metabolism and peripheral vasoconstriction in addition to behaviour while thermic fever is a sole behavioural response the present results may indicate that responses are initiated through similar intermediary steps. Since the general pattern of system as it is known in mammals pre-exists in an amphibian, there seems to be reas propose that ectothermic fever precedes that in the endotherms. Thus the present re support the theory of a common phylogenetic origin of ectothermic and endothermic f

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tion, is to large extent dependent upon pathophysiological events occurring during ...ulation. The demonstration of unperfused regions after transient cerebral ischemia ... *et al.* 1968, Chiang *et al.* 1968, Cantu *et al.* 1969 Ginsberg and Myers 1972) focused ...st on microvascular changes. Although it has been argued that the "no-reflow" phenom...n is not a common factor in the production of ischemic brain damage (Levy *et al.* ... a and b) and, although it has been shown beyond doubt that irreversible neuronal ...ges may occur in the absence of circulatory impairment (Salford *et al.* 1973 a and b, ... *et al.* 1975 a and b) postischemic microcirculatory disturbances must be considered ...he possible mechanism, contributing to give irreversible cell damage in "transient" ...mia.

...has been shown by several investigators (Baldy-Moulinier 1970 Hossmann *et al* 1973 ...er *et al* 1975) that prolonged periods of reduction in blood flow may occur following ...ient cerebral ischemia. Although it is generally held that the cerebral metabolic rate for ...en (CMR_{O_2}) is depressed during the initial postischemic recovery period (Lang *et al.* ... Snyder *et al.* 1975 Hossmann *et al* 1976) the reduction of cerebral blood flow may at ...in some instances, represent a true hypoperfusion as indicated by a low cerebral venous ...en content (Snyder *et al* 1975).

... previous communications we have shown that, in the rat, there is extensive recovery ...erebral cortex concentrations of high energy phosphates, glycolytic metabolites, citric ... cycle intermediates, associated amino acids, and ammonia after a 30 min period of ...plete cerebral ischemia irrespective of the type of anesthesia used (Nordström *et al.* ...). Following pronounced, incomplete ischemia of 30 min duration, however a com... this restitution of cerebral energy metabolism was obtained in deeply anesthetized ...enobarbital 150 mg kg⁻ *i.p.*) but not in superficially anesthetized (70% N_2O) rats ...rdström *et al* 1977 b). Since both groups of animals showed a similar degree of meta...ic perturbation during ischemia (Nordström and Siesjö 1977) it seemed likely that pheno...bital afforded protection by other mechanisms than by preventing energy depletion in ...emic cells. The objectives of the present investigation were (1) to study if the protective ...cts of barbiturate anesthesia could be attributed to a decrease in CMR_{O_2} during the ...ial recovery period, and (2) to investigate whether regional cerebral blood flow was ...sistently higher in barbiturate-anesthetized animals during the recirculation period.

Methods

...ty-seven male Wistar rats (270–300 g) with free access to tap water and rat pellets until operation were ...d in these expts. The animals were anesthetized with either 70 N_2O or phenobarbital (150 mg kg⁻¹ ...). In the nitrous oxide group, anesthesia was induced with 2 halothane and, following tracheotomy ...animals ...ere kept artificially ventilated on gas mixture containing 70 N_2O and 30 O_2. After ...cheotomy the animals in the phenobarbital group were ventilated with 70 N_2 and 30% O_2. All animals ...re given tubocurarine chloride (1 mg kg⁻¹) and heparin (100 I U). Rectal temperature was ...t close to 37°C by intermittent heating. A fall in brain temperature during the ischemic period was ...vented by placing heating bulb over the head at pre-determined distance.

Control values for regional cerebral blood flow (rCBF) ...ere obtained in animals anesthetized with ...her nitrous oxide or phenobarbital. In these animals one femoral artery and one femoral vein were ...nulated. I... all animals made ischemic, both femoral arteries and both veins ...ere cannulated. Arterial ...ood pressure was continuously recorded in all animals. Anaerobic sampling of arterial blood for measure...ments of P_{O_2}, P_{CO_2} and pH was performed at regular intervals.

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Postischemic Cerebral Blood Flow and Oxygen Utilization Ra[illegible] in Rats Anesthetized with Nitrous Oxide or Phenobarbital

By

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Abstract

NORDSTRÖM C. H and S REHNCRONA *Postischemic cerebral blood flow and oxyge[illegible] tion rate in rats anesthetized with nitrous oxide or phenobarbital* Acta physiol. sca[illegible] *101* 230–240

The present experiments were undertaken to measure postischemic regional cerebral blood flo[illegible] nd oxygen utilization rate (CMR_{O_2}) in rat anesthetized with either 70 % N_2O or phenobarbital ([illegible] kg^{-1}). I previous studies we have found that extensive rest tution of cerebral energy metabo[illegible] after 30 min of complete cerebral ischemia irrespective of the type of nesthesia used. Following [illegible] pronounced incomplete ischemia, however comparable restitution of cerebral energy state wa[illegible] in deeply anesthetized (phenobarbital 150 mg kg^{-1}) but n t in superficially anesthetized ([illegible] rats. The objectives of the present i estigation were (1) to study whether postischemic cerebral [illegible] was higher in barbiturate-anesthetized animals duri g the initial recirculation period, and (2) to [illegible] if the protective effects f phenobarbital previously observed co ld be attributed to a decrease [illegible] In both groups of animals a considerable variability in postischemic rCBF was observed betwe[illegible] animals. However no signs of gross inhomogeneity i blood flow were found and no consistent [illegible] in flow alues between the two groups of animals were observed Since the measured postischem[illegible] were identical in both groups of an mals and since cerebral venous oxygen contents ere above [illegible] results leave little support t the assumptio that, the present model of transient, incomple[illegible] ischemia failure of recovery of cerebral metabol sm (N_2O group) is primarily due to mpaired rec[illegible] nor do they indicat that the protective effects of barbiturates is due t thei abil ty to reduce rate [illegible] energy utilization

During the last few years a substantial amount of evidence has accumulated, demo[illegible] that a majority of cortical neurons restitute energy metabolism and basal neurophys[illegible] parameters after prolonged cerebral ischemia (15–60 min Hinzen *et al* 1972, H[illegible] and Kleihues 1973 Ljunggren *et al* 1974 b, Folbergrová *et al* 1974 Hossmann and [illegible] mann 1974 Zimmermann and Hossmann 1975 Kleihues *et al* 1975 Marshall *et* [illegible] Nordström *et al* 1977 a) In contrast clinical experience as well as a number of expe[illegible] studies have shown that following, for example cardiac arrest signs of irreversi[illegible] damage may develop even if the primary ischemia only last for 4–6 min (*e.g.* Cole and [illegible] 1956). Possibly the ultimate brain damage that is the end res ult o nly a few mi[illegible]

rCBF (ml g⁻¹ min⁻¹) in cerebral cortex (mean of six regions) and five subcortical regions in animals anesthetized with 70% N_2O or phenobarbital (150 mg kg⁻¹) during control conditions and after 5 and 15 min of ischemia. The values are means ± S.E.

	Control N_2O (n = 5)	Ischemia N_2O		Control Phenobarbital (n = 3)	Ischemia Phenobarbital	
		5 min (n = 3)	15 min (n = 9)		5 min (n = 3)	15 min (n = 3)
	0.54 ±0.09	0.38 ±0.17	0.34 ±0.07	0.44 ±0.04	0.37 ±0.09	0.36 ±0.03
	0.42 ±0.07	0.45 ±0.11	0.30 ±0.07	0.44 ±0.04	0.38 ±0.08	0.32 ±0.04
	0.76 ±0.18	0.20 ±0.07	0.22 ±0.05	0.43 ±0.06	0.24 ±0.05	0.25 ±0.02
[illegible]	0.70 ±0.09	0.22 ±0.09	0.21 ±0.05	0.39 ±0.03	0.24 ±0.06	0.26 ±0.05
	0.80 ±0.13	0.09 ±0.03	0.12 ±0.04	0.44 ±0.04	0.10 ±0.02	0.12 ±0.01
	0.83 ±0.11	0.04 ±0.01	0.04 ±0.01	0.40 ±0.04	0.04 ±0.01	0.08 ±0.03
	1.00 ±0.08	0.05 ±0.01	0.04 ±0.01	0.42 ±0.03	0.05 ±0.01	0.04 ±0.01

Results

l values for rCBF

l values for rCBF in animals anesthetized with either nitrous oxide or phenobarbital wn in Table I. Since no differences in flow were observed between the 6 different l regions the rCBF values are pooled. In animals anesthetized with nitrous oxide, flow was less in subcortical and brain stem regions. The values obtained are similar e previously measured with the same technique (Nilsson and Nordström 1977). In l, the results confirm those previously obtained with the autoradiographic techniques tu et al 1955 Reivich et al. 1969). During phenobarbital anesthesia, cortical blood as reduced to 40–50% of normal. The values obtained are close to those measured he Kety and Schmidt technique (Nilsson and Siesjo 1975). The reduction of rCBF phenobarbital anesthesia was most prominent in regions with a high proportion of natter (cf Freygang and Sokoloff 1958).

during ischemia

I also gives the rCBF in animals subjected to 5 and 15 min of ischemia. There were main findings. First, there was a very pronounced fall in cerebral cortex and caudate um, somewhat less marked reduction in thalamus, mesencephalon and cerebellum, moderate reductions in pons and medulla oblongata. These results reflect the charac ics of the ischemic model, e to drastically reduce flow to cortical regions while main- ng perfusion to brain stem regions. Second, there was a very small variation in rCBF een different cortical regions, and different animals. In the report by Eklof and Siesjo

Incomplete ischemia

Pronounced, incomplete cerebral ischemia was induced with a technique similar to that described b[illegible] and Siesjö (1972, see also Nordström and Siesjö 1977). Both common carotid arteries were [illegible] the vagi nerves were carefully separated from the arteries. Following operation the animals were [illegible] disturbed for 20–30 min in physiological steady state. Incomplete cerebral ischemia was induced by rubber-coated clamps on both carotid arteries and reducing the arterial blood pressure to 5[illegible] This was performed by usi g an automatically pressure-controlled syringe [illegible] extracted blood fr[illegible] femoral artery and when necessary reinfused the previously extracted blood into one of the femoral veins. Recirculation of the brain was started by removal of both clamps and simultaneous infusion of blood Withi 1 min after the start of the recirculation, systemic arterial blood pressure ex[illegible] 100 mmHg in all animals and, within a few minutes, blood pressure reached 140–150 mmHg blood pressure was never allowed to fall below 120 mmHg. During the initial recirculation period amounts of sodium bicarbonate were given I and the ventilation volume was adjusted to for onrespiratory acidosis and hypercapnia, respectively Arterial P_{O_2} and P_{CO_2} were kept abo[illegible] mmHg and close to 40 mmHg, respectively

Regional cerebral blood flow (CBF)

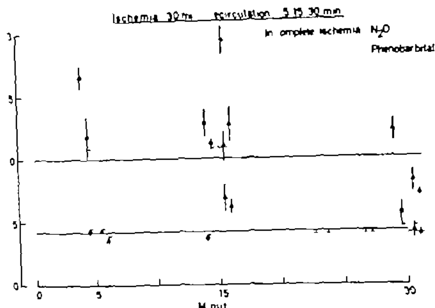
Regional cerebral blood flow was measured with ^{14}C-ethanol as a diffusible tracer (Eklöf and Sesi[illegible] Eklöf *et al* 1974) About 70 / Ci of the radioactive tracer was dissolved in physiological saline and i[illegible] L for 30 s. Arterial blood was sampled in glass capillaries every 3 s during the infusion [illegible] after the last sample had been taken, 1 ml of a saturated KCl solution was rapidly injected i[illegible] decapitated and the head was frozen in liquid nitrogen. The heads were kept at −80°C until [illegible] in refrigerated glove box at −14 C. Six cortical and 6 subcortical regions were dissected, [illegible] dded to scintillation vials and subsequently counted with the blood samples in Nuclear Chica[illegible] scintillation counter The partition coefficient for ^{14}C-ethanol was derived from Eklöf *et al.* (1974

rCBF was measured in animals anesthetized with either 70% N_2O or phenobarbital (150 mg during control conditions, after 5 nd 15 min of ischemia and after 5, 15 and 30 min of recirculation[illegible] ing ischemia of 15 or 30 min d ration. In one gro p of nimals (30 min of recirculation) cerebral [illegible] blood was taken from the superior sagittal sinus (see below) for measurement of the venous oxygen [illegible] and for calculation of arterio-venous differences in oxygen content

Cerebral metabolic rat for oxygen

For quantitative estimation of postischemic cerebral blood flow nd metabolic rate for oxygen (C[illegible] a modification f the Kety and Schmidt technique (1948) was used, as described by Norberg and [illegible] (1974). Cortical venous blood was sampled n glass pipettes by puncturing the superior sagitta[illegible] previously exposed by a burr hole. The animals were saturated with ^{133}Xenon via the insufflated [illegible] ture from rubber balloon, co taining about 10 μC of ^{133}X n either 70% N O and 30% O_2 or [illegible] and 30% O_2 Saturation was started 15 min before induct on of the ischemia and continued [illegible] ischemia d recirculation period Desaturation was started by disconnecting the rubber ball[illegible] artificial ventilation being continued with the original gas mixture The rats were desaturated for [illegible] During this period rterial and venous blood samples were withdraw at regular intervals for de[illegible] tion of the ^{133}X activity Samples for determination of oxygen content n arterial and cerebral blood were taken at the end of the saturation period nd after 5 and 10 min of desaturation. T[illegible] method requires that (1) all parts of the tissue are saturated w th ^{133}Xe before desaturation, (2) the insignificant contamination of venous blood from extracerebral so rces, and (3) cerebral blood constant during the period of desat ration. Therefore, ll animals were discarded if the arterial and venous ^{133}Xe activities differed after saturat or desaturation, f the arterio-venous differ[illegible] oxygen content (AVD_{O_2}) d ffered by more tha 10%. Blood f determination of ^{133}Xe activity and content was sampled in 50 μl glass capillaries with both ends drawn to thin tips Blood pressure constant during the measurements, usi g the automatically controlled syri ge. Arterial P_{O_2}, P_{CO_2} were determined bef re and during desaturation.

The ^{133}Xe activity of the blood samples was measured Wallac gamma counter CBF as ca[illegible] from the integrated arterial and cerebral venous desaturation curves (Norberg and Siesjö 1974). tissue/blood coefficient for ^{133}X of 0.8. The arterial and venous ygen contents were measured method of Fabel and Lübbers (1964) as described by Borgst öm *et al.* (1974). CMR_{O_2} was calcu[illegible] the product of CBF nd AVD_{O_2}



... Individual cortical blood flow values (mean ± S.E. for 6 cortical areas) obtained after 5, 15 and ... of recirculation following 30 min of pronounced incomplete ischemia in animals anesthetized with 70 ... N$_2$O or phenobarbital (150 mg kg^{-1}). Present control values for cortical blood flow during ... oxide (1.00 ... 0.08 ml g^{-1} min^{-1}) and phenobarbital (0.42±0.03 ml g^{-1} min^{-1}) anesthesia are

...cal differences were small ... each animal. In a few animals a "reactive hyperemia was ...rved during the first 5 min of recirculation. In the majority of animals, however cortical ...d flow was between 0.3 and 0.7 ml g^{-1} min^{-1} irrespective of the type of anesthesia. ... results demonstrate that, in animals anesthetized with phenobarbital, postischemic ...F values are close to those measured in the control situation. In contrast, rCBF in nitrous ...de-anesthetized animals was usually below control and, in some animals, the values were ... 30–40 ... of control.

...F during recirculation: 30 min of ischemia

... 2 demonstrates cortical blood flow (mean ± S.E. for 6 different cortical areas) after 5 ... and 30 min of recirculation in animals anesthetized with either nitrous oxide or pheno...bital. Again the variation in cortical blood flow between different animals was con...erable. However variations between different cortical areas were minor. Apart from the ... that there were some very high rCBF values also after 15 min of recirculation, the results ...re similar to those observed after 15 min of ischemia. Thus, in nitrous oxide-anesthetized ...mals there were some rCBF values that were only 30–40% of control.

...tischemic oxygen utilization

... normal rates of oxygen consumption in the brain, ... reduction in CBF to below about ...% of control is usually considered inadequate for maintained energy production. It thus

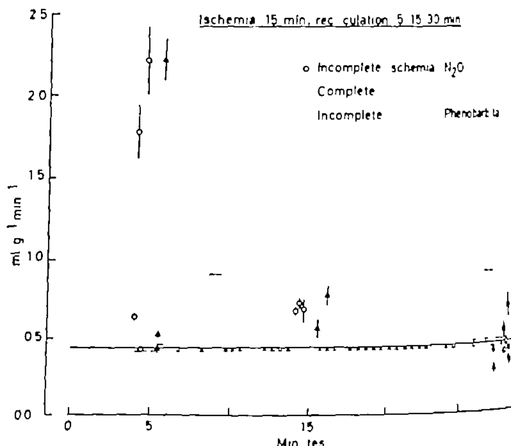


Fig. 1 Individual cortical blood flow values (mean ± S E. for 6 cortical area) obtained after 5, 15 r min of recirculation follow g 15 min of pronounced incomplete ischemia in animals anesthetized either 70 % N_2O or phenobarbital (150 mg kg). For comparison, cortical blood flow also obta 2 animals (70 % N O) after 30 min of recirculation following 15 min of complete ischemia are ad Present control values for cortical blood flow during itrous xide (1 00 ± 0.08 ml g min) and p ba bital (0 42 ± 0.03 ml g min) nesthesia are gi en.

(1972) the variations in cortical blood flow were pronounced. With the present slight r fication of the experimental technique (a further reduction of blood pressure to 50 m such variations were not observed However this does not exclude the possibility c homogeneity of blood flow at the capillary level Third, in all animals, whether anesthe with nitrous oxide or phenobarbital cortical blood flow was reduced to about 0 05 ml min *i e* to about 5% of control in nitrous oxide-anesthetized animals and about 10 those given phenobarbital.

rCBF during recirculation. 15 min of ischemia

rCBF was measured after 5 15 and 30 min of recirculation in animals anesthetized either nitrous oxide or phenobarbital Fig. 1 gives the cortical blood flow (mean ± S.E 6 different cortical areas) obtained in 27 animals. For comparison the figure also s postischemic cortical blood flow in two animals subjected to 15 min of complete cer ischemia (induced by elevation of the intracranial pressure above the arterial blood pre See Ljunggren *et al* 1974 a, Nordström *et al* 1977 a) As demonstrated in the figure reg

..s III gives the physiological parameters in these animals, as well as the arterio-venous ...rences for oxygen, the cerebral blood flow and the calculated CMR_{O_2}. The differences ... and CMR_{O_2} between animals anesthetized with nitrous oxide and phenobarbital ...ot significant. During physiological conditions, phenobarbital anesthesia reduces ...nd CMR_{O_2} by 40-50% when compared to nitrous oxide control values (Nilsson and 1975). Thus, in phenobarbital-anesthetized animals neither CBF nor CMR_{O_2} appears ...educed during the initial recovery period following 15 min of ischemia. In contrast, ...s under 70% N_2O had their CMR_{O_2} reduced to about 60% of control.

Discussion

...l previous investigations ha.e described the postischemic cerebral blood flow (Hoss... et al. 1973, Hossmann and Kleihues 1973, Snyder *et al.* 1975 Nemoto *et al.* 1975) ...xygen utilization rate (Lang *et al* 1972, Snyder *et al* 1975, Hossmann *et al.* 1976). ...eral, correlation between postischemic hyperemia during the initial recirculation ... and functional recovery has been found (Hossman and Kleihues 1973, Osburne and ...r 1975, Snyder *et al.* 1975). However since oxygen utilization rate may be considerably ...ned during this period (Hossmann *et al* 1976) it may be questioned whether this ...emia is necessary for restitution.

...: prolonged period of increase in cerebral blood flow frequently seen after ischemia ...ually been explained by vasoparalysis due to lactacidosis (Zwetnow *et al.* 1968, Häggen... *al.* 1970 Kogure *et al.* 1970, Langfitt *et al.* 1965 Lassen 1966) but several factors are ...bly involved. Thus, it is known that tissue hyperosmolarity is an important mediator ...odilatation in skeletal muscle (Mellander *et al.* 1967 Lundvall 1972) and in secreting ...ry glands (Lundvall and Holmberg 1974), and infusion of hypertonic solutions have ...shown to affect vascular smooth muscles in brain (Johnston and Harper 1973 Wahl ... 1973). Certainly release of vasoactive substances during cerebral ischemia may also ...ondly affect vascular tone. A reduction of postischemic cerebral blood flow has been ...uted a combination of pathophysiological changes (1) aggregation of intravascular ...d corpuscles and increased viscosity (2) neuronal and glial swelling, and (3) capillary ...thelial swelling (Ames *et al.* 1968, Chiang *et al* 1968, Cantu *et al.* 1969 Fischer and ...s 1972, Fischer *et al.* 1975). Although it has been maintained that microvasculary ...rbances are not a common factor in the production of ischemic brain damage (Levy ...l. 1975 a and b) they must nevertheless be considered since ...ggregation of blood cor...cles, leading to capillary obstruction, has been observed to occur *in vivo* (Eriksson and ...nder 1972) and since the "no-reflow" phenomenon has been described also in other ...ans (Flores *et al.* 1972, Franklin *et al.* 1974 Glaumann and Trump 1975). Altogether ...e complex mechanisms suggest the possibility of inhomogeneous blood flow following ...bral ischemia.

...Most techniques used to measure cerebral blood flow are only valid if the flow is homo...eous. I... the present investigation, this problem was approached by using two different ...thods for measuring cerebral blood flow and by combining the results with data obtained ...measuring cerebral energy metabolites (Nordström *et al.* 1977 a and b).

TABLE II Individual values for arterial and venous oxygen content, arteriovenous oxygen dif[...] cortical blood flow (mean ± S.E. for 6 regions) after 30 min of recirculation, follow[...] of ischemia in animals anesthetized with 70% N_2O or phenobarbital (150 mg kg^{-1}).

Anaesthesia	T_{aO_2} µmol ml^{-1}	T_{vO}	AVD_{O_2}	Cortical blood flow ml g min^{-1}
N O	10 45	9.26	1 19	0.56 ±0.09
	11.55	7 75	3.80	0.36 ±0 02
	8 97	7.80	1 17	1.21 ±0.09
	12.46	10 08	2.38	0.53 ±0 01
Phenobarbital	9 16	7.33	1 83	0.71 ±0.03
	10 41	9 94	0.47	0.81 ±0.09
	11 99	9 33	2.66	0.40 ±0.03
	10.78	8 18	2.60	0.42 ±0.06

became of crucial importance to find out whether or not postischemic blood flo[...] quate for the energetic requirements In order to study this problem, venous oxyg[...] was measured and CMR_O was determined.

In animals exposed to 30 min of ischemia arterial and cerebral venous oxyg[...] were measured following 30 min of recirculation. Individual values are given i[...] There were some high arterial oxygen contents, probably due to hemoconcentra[...] ever since cerebral venous oxygen contents were higher than normal the resul[...] indication that blood flow was insufficient for the requirements.

A quantitative measurement of cerebral blood flow and oxygen utilization rat[...] was performed in 10 animals after about 30 min of recirculation following 15 min c[...]

TABLE III Physiological parameters, cerebral blood flow (CBF) and calculated cerebral metal[...] oxygen (CMR_{O_2}) after 30 min of recirculation, following 15 min of ischemia in [...] esthetized with 70% N_2O or phenobarbital (150 mg kg^{-1}). The values are means [...]

	N_2O (= 5)	Phenobarbital (= 5)
MABP mmHg	135±4	130±3
Temp, °C	37 1±0 3	36 9±0.1
P_{aO_2}, mmHg	117±14	114±14
P_{aCO_2}, mmHg	38.0±1 4	40 9±4..
pH	7 131±0 063	7..26±0.042
T_{aO_2}, µmol ml^{-1}	11.2±0.8	10.5±0.3
T_{vO_2} µmol ml^{-1}	6.3±1.2	6 1±0.3
AVD_{O_2}, µmol × ml^{-1}	4.8±0.5	4 4±0.3
CBF ml g^{-1} × min^{-1}	0.60±0.07	0.71±0 07
CMR_{O_2}, µmol × g^{-1} min^{-1}	2.8±0.1	3 1±0.2

III gives the physiological parameters in these animals, as well as the arterio-venous for oxygen, the cerebral blood flow and the calculated CMR_{O_2}. The differences d CMR_{O_2} between animals anesthetized with nitrous oxide and phenobarbital ignificant. During physiological conditions, phenobarbital anesthesia reduces CMR_{O_2} by 40–50% when compared to nitrous oxide control values (Nilsson and). Thus, in phenobarbital-anesthetized animals neither CBF nor CMR_{O_2} appears ed during the initial recovery period following 15 min of ischemia. In contrast, der 70% N_2O had their CMR_{O_2} reduced to about 60% of control.

Discussion

revious investigations have described the postischemic cerebral blood flow (Hoss-*l.* 1973 Hossmann and Kleihues 1973 Snyder *et al.* 1975 Nemoto *et al.* 1975) n utilization rate (Lang *et al.* 1972, Snyder *et al* 1975, Hossmann *et al* 1976). , a correlation between postischemic hyperemia during the initial recirculation d functional recovery has been found (Hossman and Kleihues 1973, Osburne and 75 Snyder *et al.* 1975). However since oxygen utilization rate may be considerably during this period (Hossmann *et al.* 1976) it may be questioned whether this ı ıs necessary for restitution.

olonged period of increase in cerebral blood flow frequently seen after ischemia ly been explained by vasoparalysis due to lactacidosis (Zwetnow *et al.* 1968, Häggen-1970 Kogure *et al* 1970, Langfitt *et al.* 1965 Lassen 1966) but several factors are involved. Thus, it is known that tissue hyperosmolarity is an important mediator latation in skeletal muscle (Mellander *et al* 1967 Lundvall 1972) and in secreting glands (Lundvall and Holmberg 1974), and infusion of hypertonic solutions have wn to affect vascular smooth muscles in brain (Johnston and Harper 1973 Wahl 73). Certainly release of vasoactive substances during cerebral ischemia may also tly affect vascular tone. A reduction of postischemic cerebral blood flow has been d a combination of pathophysiological changes. (1) aggregation of intravascular rpuscles and increased viscosity (2) neuronal and glial swelling, and (3) capillary ial swelling (Ames *et al.* 1968, Chiang *et al.* 1968, Cantu *et al.* 1969 Fischer and 972, Fischer *et al* 1975) Although it has been maintained that microvasculary rces are not a common factor in the production of ischemic brain damage (Levy 75 a and b) they must nevertheless be considered since aggregation of blood cor leading to capillary obstruction, has been observed to occur *in vivo* (Eriksson and r 1972) and since the "no-reflow" phenomenon has been described also in other (Flores *et al.* 1972, Franklin *et al* 1974 Glaumann and Trump 1975). Altogether omplex mechanisms suggest the possibility of inhomogeneous blood flow following l ischemia.

t techniques used to measure cerebral blood flow are only valid if the flow is homo-s. In the present investigation, this problem was approached by using two different ls for measuring cerebral blood flow and by combining the results with data obtained during cerebral energy metabolites (Nordström *et al.* 1977 a and b).

Our previous results demonstrate that following severe, incomplete ischemia of duration, extensive recovery of cerebral metabolic state is observed in animals anesthe with 150 mg × kg of phenobarbital, but not in those maintained on 70 % N_2O results raise the following questions. *First* is failure of recovery in nitrous oxide-anesthe animals caused by vascular factors? Second, is the protective effect of phenobarbital du reduction in metabolic rate to levels that can be covered by the (reduced) postischemic rate?

When recirculated following 15 min of ischemia most animals had regional blood flow values between 0 3 and 0 7 ml g min. We recall that the lowest represent only about 30 % of control (see Results). These values are in reasonable agre with those obtained with the Kety and Schmidt technique. Since only 3 out of 24 (70% N_2O) *showed incomplete restitution of cerebral energy metabolism following* period of ischemia (see Nordström *et al* 1977 b) we may conclude that no cortical remained unperfused and that the low cerebral blood flow measured was sufficie metabolic demands. This conclusion is supported by the high venous oxygen content sured *and the reduced* CMR_{O_2} *explains why the low CBF values encountered could* the energetic requirements Since postischemic CMR_{O_2} did not differ between anesthetized with nitrous oxide and phenobarbital, the term "hyperemia" become biguous.

Following 30 min of ischemia, no animal anesthetized *with nitrous oxide showed* bolic recovery while all animals given phenobarbital showed extensive restitution of energy state (Nordström *et al* 1977 b) The results obtained in the present investigat not explain this difference. Thus, although several very low values for cortical rCBF *observed there were no consistent differences between the two groups of animals.* more all animals had cerebral venous oxygen contents above control after 30 min of culation In other words, the present results leave little support to the assumption that of recovery of cerebral metabolism in the present model of transient cerebral ische due *to impaired recirculation of ischemic tissue, nor do they indicate* that the pro effect of barbiturates is due to their ability to reduce rate of cerebral energy utilizati

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Cardiac Receptors Activated during the Hypothalamic Defence Reaction

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Abstract

Wennergren, G., P. Thorén and B. Lisander. *Cardiac receptors activated during the hypothalamic defence reaction.* Acta physiol. scand. 1977 *101* 241 246.

Increases of arterial blood pressure, cardiac inotropy and venous return seen during the hypothalamic defence reaction are likely to lead to concomitant excitation of left ventricular receptors with nonmedullated afferents. The integrated efferent pattern of response resulting from the central interaction between the defence reaction and influences from the mentioned cardiac receptors was recently analyzed. These two, mutually opposing influences on the circulation were then seen to interact in such a way as to produce an optimal cardiovascular response with respect to increases in cardiac output and blood supply to the skeletal muscles. However, direct electrophysiological recordings from nonmedullated cardiac afferents during defence area stimulation have hitherto been lacking. The present experiments, performed on chloralose-anesthetized cats and utilizing electrophysiological recordings, clearly demonstrate that the left ventricular receptors are activated by the cardiovascular readjustments induced by the defence reaction. Defence area stimulation increased the activity of these receptors, which work within a very narrow low frequency range, from 1.1 0.3 imp/s to 2.7±0.7 imp/s associated with rises in systolic blood pressure (afterload) and heart rate. Normally such receptor activation would induce considerable bradycardia and sympathetic inhibition, but particularly the reflex bradycardia is centrally suppressed by the concomitant defence area stimulation. The marked bradycardia often seen immediately upon interruption of the defence area stimulation is, however, probably to a great extent initiated from the excited ventricular receptors.

During the hypothalamic defence reaction, which is linked to situations of emotional excitement and even flight or attack, drastic readjustments of the circulation are seen. These cardiovascular changes, generally excitatory in nature, certainly lead to excitation of cardiovascular proprioceptors such as e.g. the arterial baroreceptors (e.g. Hilton 1963). However there is reason to assume that the cardiac left ventricular receptors with nonmedullated afferents also are activated during a full-fledged defence reaction (Wennergren, Lisander and Oberg 1976). This assumption seems reasonable in view of the cardiovascular changes during the defence reaction with a combination of arterial blood pressure rise, venoconstriction (Cobbold *et al.* 1964) and hence increased venous return as well as increased cardiac inotropy (Rosen 1961), all factors known to activate these cardiac receptors (*cf.* Sleight and Widdicombe 1965 Öberg and Thorén 1972, Muers and Sleight 1972, Thorén 1977).

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[illegible] opened, nonbeating heart. The spikes were counted by [illegible] spike counter device writing on the Grass [illegible]graph. The technique has been described at length elsewhere, [illegible] Öberg and Thorén (1972) and Thorén [illegible] which papers should be consulted for details.

[illegible]rin (Vitrum), 1 000–1 700 IU/kg i.v. was used as anticoagulant. Once the stimulating electrode had [illegible] correctly positioned in the D.A. muscle flow was excluded and the heparin was neutralized with [illegible]mine chloride (Vitrum), 10 mg per 1 000 IU heparin, in order to minimize bleedings in the area where [illegible] afferents were detected for recording. During that recording the cats were curarized with gallamine [illegible]iodide (Flaxedil®), 3–5 mg/kg i.v., to eliminate disturbing muscle movements.

[illegible]mental procedures and treatment of data

[illegible] D.A. was activated during concomitant recording of cardiac receptor activity and with the hypothalamic [illegible] [illegible]ode position kept unaltered from the earlier test stimulations. The hypothalamus was stimulated for [illegible] 30–40 [illegible] and the stimulation was turned off once the defence reaction was fully developed. Repeated [illegible]ions of D.A. stimulation with concomitant recording of cardiac afferent activity was performed and [illegible] kept and the D.A. was standardized manner allowed to recover between each stimulation period. [illegible] electrical noise implied by the D.A. stimulation made simultaneous recording of cardiac afferent [illegible]vity impossible. Instead, the receptor activity prevailing immediately after the D.A. stimulation was [illegible]d off, was taken to represent the induced receptor activity.

[illegible] several recordings were performed in each experiment, average values for each experiment [illegible] [illegible]lated. These averaged data were used for calculating means ± S.E. in the whole series. Each cat has [illegible] been treated with equal weight in the statistical analysis. Differences between pre- and poststimulation [illegible] were tested in paired tests. P-values < 0.05 were taken to indicate significant difference.

Results

[illegible] 1 shows an example from a representative experiment. The D.A. is stimulated wit[illegible] Hz, 1 ms, 0.2 mA, *stimulus parameters which earlier in the expt. were found to produc*[illegible] [illegible]ypical defence reaction with, in addition to the heart rate increase and blood pressure ris[illegible] [illegible]monstrated in Fig. 1, also a skeletal muscle vasodilatation. A powerful poststimulation [illegible]chycardia was seen before administration of gallamine, which besides the curare-like *effec*[illegible] [illegible]agolytic. The electrophysiological recording is from a non-medullated cardiac afferen[illegible] [illegible]h the receptor located in the left ventricle. It is seen that the cardiovascular readjustmen[illegible] [illegible]ing the defence reaction lead to a clearcut increase in the receptor activity which goes [illegible] [illegible]m about 0.4 imp/s *to 2 imp/s. The activation of the receptor by the D.A. stimulation* [illegible] [illegible]rly seen when comparing the 'silent' control *neurogram (A) with that taken immediate*[illegible] [illegible]er the end of the D.A. stimulation (B) where the *receptor has started to fire regularl*[illegible] [illegible]ilar increases in activity at defence *reaction were seen in all left* ventricular recepto[illegible] [illegible]orded from.

The results obtained *from 5* receptors in 5 cats are summarized in Table I. D.A. stimulati[illegible] [illegible]reased cardiac receptor activity *from 1.1 ± 0.3* imp/s during the control situation [illegible] [illegible] 0.7 imp/s. The *difference is statistically* significant. It should be pointed out in t[illegible] [illegible]ment that these cardiac receptors *with non-medullated* afferents work within a v[illegible] [illegible]rrow low frequency range (Öberg and Thorén 1972). At electrical stimulation of [illegible] [illegible]ferent fibres, the steepest part of the frequency-reflex response curve occurs below 4 [illegible] [illegible]berg and Thorén 1973 a, *Little, Wennergren and Öberg 1975). All the* receptors on wh[illegible] Table I is based, were found in the left ventricle which is the main location *for card*[illegible]

One question which then, of course, arises is to what extent these secondary [illegible] fluences may modify the primary centrally elicited defence reaction. When activated, cardiac receptors with nonmedullated afferents normally give rise to a strong vagal [illegible] cardia and a generalized inhibition of sympathetic tone to the vascular beds and the k[illegible]. Thus, the reflex pattern normally produced by these cardiac receptors largely oppose mainly excitatory pattern initiated from the hypothalamic defence area. An analysis of integrated response patterns resulting from interaction between the hypothalamic de[illegible] reaction and influences from cardiac receptors with nonmedullated afferents has rec[illegible] been performed (Wennergren, Lisander and Öberg 1976)

However although the assumption that the left ventricular receptors are activated [illegible] the defence reaction may seem very reasonable, direct electrophysiological recordings [illegible] nonmedullated cardiac receptor afferents during defence area (D.A.) stimulation [illegible] earlier been lacking. The present experiments therefore have been performed to invest[illegible] with electrophysiological techniques, whether and to what extent, left ventricular rec[illegible] activity is augmented during the defence reaction The results confirm the hypothesis [illegible] the receptors are activated

Methods

The experiments were performed o cats of either sex, anesthetized with chloralose (30–50 mg/kg [illegible] after induction with ether A tracheal cannula was inserted and the animals were placed on positive [illegible] sure ventilation with 100 per cent O with a tidal volume of 10–12 ml/kg b.w The vago-sympathetic [illegible] *and the carotid rteries were bilaterally cautiously freed in the neck. The* rterial baroreceptors [illegible] were left intact except the left carotid sinus which was functionally excluded due to cannulation of the common carotid artery (see below).

T counteract acidosis the cats were given n i. *infusio (0.10–0.15 ml/min) of 10–20 mEq NaH[illegible]* 100 ml dissolved in 10 per cent glucose solution (Haglund 1973) Acid–base balance as checked [illegible] out and corrections were made by adjusti g the rate of bicarbonate infusion.

A monopolar stainless steel electrode was placed in the hypothalamic D A. using a Horsley-C[illegible] apparatus Stimuli were given by constant current device as square wave pulses. The stimulation [illegible] was usually chosen t A 12 5 in the perifornical region (*f* Jasper nd Ajmone M rsan 1961)) A [illegible] vasod lat tion resulting in a fall of flow resistance by 75 per cent or more with less than 10 latency [illegible] *a test stimulation at 0.2–0 3 mA 1 ms duration and 80 Hz, was used as the criterion that the electrode* positioned i the D A The st mulation point was lso determined hist logically after formalin per[illegible] a d frozen sectioning.

When the correct position of the elect ode had been ensured the electrode was fixed to the skull with dental cement nd the cat removed from the Horsley–Clark apparatus to facilitate the prep[illegible] for the electrophysi logical recordi g from cardiac afferents.

Recording

Record ngs were made on a Grass Polygraph Model 7 B 7 D and on ABEM 5631 ultra viol[illegible] (UV) recorder

Arterial blood pressure left ventricular pressure and left atrial pressure were measured by Statham [illegible] ducers (P23AC and P23DC) connected to catheters inserted through the left *femoral* rtery left com[illegible] carotid artery nd right upper lung vein, respectively *Heart rate* w recorded by a tachograph trig[illegible] by the rapid systolic blood pressure rise *Skeletal muscle blood flow* was meas red with a drop rec[illegible] technique as the venous outflow from the calf muscles

Cardiac left ventricular receptor activit w s recorded, w th the thorax open, from non-medullated [illegible] afferent f bres dissected free in the right cervical vagus nd displayed on the UV recorder The rec[illegible] were localized to the left ventricle by thei response to aortic occlusion nd lack f response to atrial occlusion (Swan–Ganz balloon catheter 93–110, 5F). The localization wa confirmed by mechanical pr[illegible]

1 The responses to D.A. stimulation in terms of heart rate, systolic blood pressure (i.e. afterload), left atrial pressure and left ventricular receptor activity. Note the significantly increased cardiac receptor activity in response to D.A. stimulation. Data based on average values from 5 cats. Mean ± S.E. non significant.

	Heart rate beats/min	Systolic arterial blood pressure mmHg	Left atrial pressure mmHg	Left ventricular receptor activity imp/s
ol	212±5	151±11	5.6±0.4	1.1±0.3
D.A. stim.	230±6	17±11	7.0±0.8	2.7±0.7
	5	5	5	5
P	<0.05	0.001	n.s. (0.2)	<0.05

edicted by Wennergren, Lisander and Öberg (1976). In that study the interaction be- n the two influences was analysed and it was shown that a strong D.A. activation can pletely suppress the otherwise powerful reflex vagal bradycardia elicited via the ventricu- eceptors. In contrast, the inhibitory reflex influences from ventricular receptors on athetic vasoconstrictor and cardio-accelerator discharge seems to be unhampered to te with the excitatory influences from the defence area, the net effect being largely a nation of the reflex inhibitory and central excitatory influences on the peripheral sym- etic neurons.

such a way the ventricular receptors can secondarily modify the primary centrogenic nce area influences, with the net result that two essentially oppositely directed influences he cardiovascular system lead to an improved cardiovascular response in terms of a e efficient cardiac performance and enhanced blood supply to the skeletal muscles nnergren, Lisander and Öberg 1976).

Then, however, a D.A. stimulation is turned off the tachycardia seen during stimulation ually replaced by powerful rebound" bradycardia. This poststimulatory bradycardia t demonstrated in the sequence show in Fig. 1 because of the gallamine administration causes vagal efferent blockade, but was seen before this administration. It has been gested that this bradycardia should be a baroreceptor reflex effect elicited by the in- sed arterial pressure and augmented heart rate. Due to the efficient suppression from the nce area of the baroreflex bradycardia, this reflex effect should, however not develop l the D.A. stimulation is turned off (Hilton 1963). However bradyarrhythmias following vation of hypothalamic pressor areas are still present after complete denervation of all rial baroreceptors (Lisander Jaju and Wang 1975), suggesting that the poststimulation dyarrhythmias may also be reflexly induced via cardiac receptors.

The present demonstration that the unmyelinated cardiac ventricular receptors are indeed vated as a result of D.A. stimulations makes it likely that the poststimulation brady- dia is initiated from such receptors, the more so as they can elicit a much more potent inotropic bradycardia than the baroreceptors (Öberg and Thorén 1973 b). The reflex dycardia component from the ventricular receptors is suppressed during D.A. stimulation t as effectively as that from the arterial baroreceptors, and should therefore not appear il the D.A. stimulation is interrupted, as was also clearly shown by Wennergren, Lisander Öberg (1976).

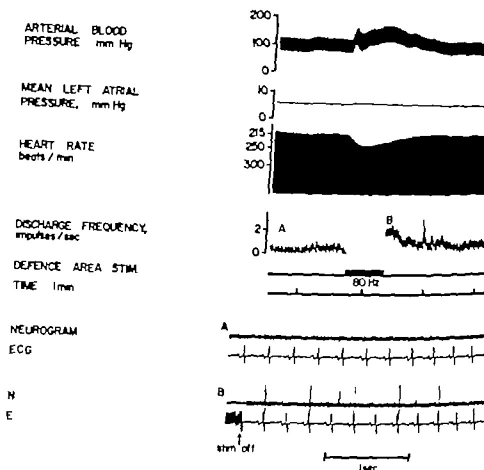


Fig. 1 The effects of D.A. stimulation (80 Hz, 1 ms, 0.. mA) on the activity of a left ventricular r... signalling in a non-medullated afferent Note that the defence reaction, with the typical increase ... rate and arterial blood pressure, leads to a clear excitation of the ventricular receptor The spike fre... increases from in average 0.4 imp/s before D.A. stimulation to about 2 imp/s immediately after th... stimulation is turned off Due to the prominent stimulation artefact the input of the nerve ampli... shortened during the stimulation period. The lower part of the Figure show neurograms from the... fiber in the control situation (A), where no spikes appear during the recording segment and imme... post D.A. stimulation (B), where the receptor has started to fire regularly at about 2 imp/s.

receptors with non-medullated afferents in the cat (Thorén 1977) The ventricular re... activation was accompanied by a rise in systolic blood pressure (i.e. afterload), from ... 11 mmHg to 217 ± 11 mmHg and an increased heart rate from 212 ± 5 to 230 ± 6 beats... With regard to left atrial pressure which rather well reflects also left ventricular enddia... pressure, there was a tendency towards an increased preload the pressure increasing f... 5 6 ± 0 4 to 7 0 ± 0 8 mmHg, though this difference is not significant.

Discussion

The present experiments have demonstrated with electrophysiological recordings, that left ventricular cardiac receptors, signalling in non-medullated afferents, are activated in the course of the cardiovascular readjustments induced by the hypothalamic defence ...

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Effects of Low Tissue Temperature on Peripheral Vascular Control Mechanisms

By

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Abstract

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…ence of low temperatures on vascular effects produced by adrenaline, noradrenaline and vasoconstrictor … stimulation was studied in the feet of ducks. Observations …ere made on isolated preparations as … as on intact ducks with one or both feet immersed in ice-water. Isolated preparations …ere perfused … physiological solution and information on changes in vascular resistance obtained by direct measure… of flow changes during perfusion at constant pressure or by measuring changes in perfusion pressure … perfusion at constant flow. In intact ducks changes in blood flow …ere recorded as changes in digital …aneous tissue temperature. The study revealed that in the duck foot … relatively large fraction of the …tance to blood flow is found in the arteries of the proximal part of the foot. During cooling of the … the influence of vasoconstrictor nerve stimulation and low doses of adrenaline and noradrenaline on …ular resistance is gradually lost, and it is negligible at temperatures below 8°C. At these low tempera… the blood vessels stay dilated and blood flow through the web seems to be determined by the digital …rial blood pressure.

…he vascular changes induced by cold exposure of local tissues, particulary extremities, …e been studied by many investigators (Lewis 1930, Grant and Bland 1931 Kramer and …ultze 1948, Folkow *et al.* 1963). Initially the exposed vessels show construction, followed … vascular relaxation if the cooling is continued. The mechanisms involved in the vascular …ponse to cold are probably complex (Folkow *et al.* 1963), and their nature has not been …ly explained.

Many birds are exposed to low ambient temperatures during winter In such birds total …dy heat economy may require that blood flow through peripheral tissues like the feet be …minished at a time when high blood flow is necessary to prevent local tissue freezing. This …ses the question of the relative importance of central and local vascular control in cold-…posed tissues.

The present paper reports on expts. into the efficiency of local vascular control mech-

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During physical exercise similar cardiovascular changes are seen as in the defence rea with increases in both inotropism and in pre and afterload. It has been postulated the ventricular receptors are activated by the cardiovascular adjustments during exercise (P and Widdicombe 1965 Thorén 1972) and the present findings are in line with such a pos

This study was supported by grants from the Faculty of Medicine Uni ersity of Göteborg and the Swedish Medical Research Council (14X-4249 and 14X-4769).

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...fect of injection of noradrenaline (NA, 0.1 μg, 0.02 μg and 0.01 μg) and of electrical stimulation (S) and nerve trunks (N) on perfusion pressure in the isolated duck foot perfused at 24°C. Note ...ical stimulation of both artery and nerve trunks (S + N) in this preparation produced an effect ...that of the lowest dose of noradrenaline (0.01 μg). The effect of stimulating the nerve trunks (N) ...ery (S) alone is less.

...aused a rise in perfusion pressure. Such stimulation also caused movement of the ... The latter effect was prevented by adding d-tubocurarine to the perfusion fluid ...g/ml), which in turn did not abolish the rise in perfusion pressure. A similar rise in ...n pressure was also produced by periarterial stimulation. Simultaneous stimulation ...s and artery wall gave a summation of the effects (Fig. 1). Injections of single doses ...drenaline (0.01–0.1 μg) into the perfusion fluid produced a dose-dependent rise in ...n pressure. In one preparation, stimulation of the artery at 19°C increased the per... ...ressure to the same extent as did 0.01 μg noradrenaline (Fig. 2, lower tracing). When ...t was cooled to 11°C doubling of the noradrenaline dose produced a response about ... that caused by periarterial stimulation (Fig. 2, middle tracing). At still lower tem...es (4°C) the effect of periarterial stimulation was absent or negligible, and low doses ...drenaline (0.01–0.02 μg) had also no effect. Higher doses of noradrenaline (0.1 μg) ...oduced weak but longlasting rise in perfusion pressure (Fig. 2, upper tracing) ...arming of the preparation, it was shown that changes in the response to noradrenaline ...ctrical stimulation with cooling were reversible. The influence of temperature on the ...of nerve trunk stimulation was the same as that on the periarterial nerve stimulation. ...pendent of the effects of electrical stimulation and noradrenaline there was a rise in ...rfusion pressure with decreasing temperature. Cooling from 25°C to 5°C caused a rise ... mmHg. The rise was reversed by rewarming the preparation.

...perature measurements in intact ducks. With one foot immersed in ice-water (0°C) up ...proximal end of the tarsal–metatarsal section and the other foot kept in air (22–23°C) ...eet showed slow irregular temperature fluctuations which followed each other in time ...irection, but were often different in magnitude. In the ice water immersed foot the ...rature measured subcutaneously on the medial side in the proximal part of the third ...usually fluctuated within the range of 2 to 9°C. This temperature range was reached ...5 min after immersion. The temperature of the foot in air usually stayed above 28°C, ...as in some birds only 2–3°C lower than the cloacal temperature of 40–41.5°C. Spon...ous bilateral temperature fluctuations were also seen when both feet were exposed to ...ir ambient temperatures (air or ice-water), but in that case the fluctuations of the two ...occurred within the same temperature range.

...ood pressure recording. Mean aortic blood pressure ranged between 135 and 220 mmHg. ...blood pressure in the digital arteries generally followed the aortic blood pressure, but

anisms in the feet of ducks at temperatures normally encountered during cold exp... Vascular responses to sympathetic nerve stimulation and exogenous noradrenaline at ... temperatures were studied in isolated perfused feet. Observations were also made on ... flow changes in the feet of intact ducks after intravascular administration of adrenaline ... noradrenaline.

Materials and Methods

15 domestic ducks (*Anas boscas* b.w. 2.5–3.0 kg) were studied. All expts. were conducted during the ... months. The ducks were obtained from a local farmer shortly before being used. They had been k... sheltered housing where air temperatures varied from about −5 to 10°C.

For perfusion of the isolated leg of the duck the foot artery was cannulated in the tarsal-... region after amputation of the leg. Amputation was performed under sodium pentobarbital an... (30 mg/kg b.w.). The polyethylene catether used for the cannulation was filled with heparinized ... the vascular bed was immediately flushed of blood. The two large mixed nerve trunks running ... the main artery in the tarsal–metatarsal region were isolated to allow electrical stimulation. A ... periarterial stimulating electrode was secured around the artery distal to the cannulation site. T... was perfused at constant flow by means of peristaltic pump, using a physiological solution (... o ygenized with a gas mixture containing 95% O_2 and 5% CO_2. The perfusion pressure was initially ... to about 35 mmHg by adjusting the pump. Flow of perfusate at this pressure was 10–1... ml/min ... to 32–40 ml per 100 g tissue). A higher initial perfusion pressure (60 mmHg) was tried, but this ... alter the nature f the results and had the ad erse effect of increasing fluid accumulation in the ...

During perfusion the foot was immersed in water bath. The bath was gradually cooled from 25° ... about 2 C by ddition of crushed ice. The temperature of the perfusion fluid was adjusted to equal ... the bath.

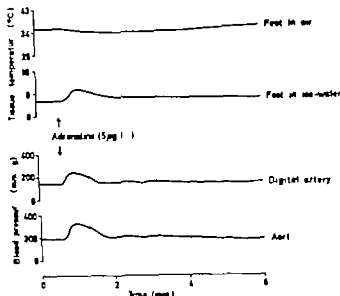
Electrical stimulation (10 V 10 Hz, 5 ms duration) of nerve trunks and periarterial nerves, ... was performed at various temperatures. The vascular response to these electrical stimulations—which ... found to be of supramaximal i tensity at all temperatures where a response could be elicited—was com... with the response to intravascularly administered noradrenaline. Noradrenaline, (0.01–0.1 μg) in ... of 0.1–0.25 ml of physiological saline, was i jected i to the perfusion fluid (through the ... of the ... shortly before it entered the foot artery. Changes in perfusion pressure monitored by electroman... reflected the magnitude of the vascula responses.

Experiments on intact ducks were performed under sodium pentobarbital anesthesia (30 mg/kg ... given i.v. supplemented by smaller doses as required). The ducks were suspended in a mesh net i... prone position, with their feet protruding to allow immersion in mixture of crushed ice and water ... temperature of the room was 2... 23 C. Aortic and digital arterial blood pressures ... ere measured ... electromanometers co nected to polyethylene catheters. The aortic blood pressure was obtained by ... ing the catheter through the brachial artery. Digital arterial blood pressure as recorded from the ... digital artery in the proximal third of one of the digits. Drugs, dissolved in physiological saline, ... administered through a catheter in a brachial vein in volumes of 1 ml over period of about 5 ... blood pressure was calculated as two-third of the diastolic pressure plus one-third of the systolic pre... Cloacal and subcutaneous foot temperatures were determined by mea s of thermocouples. All data ... recorded continuously over periods p to 6 h.

The vascular beds of the feet of two intact nesthetized ducks were studied in partly isolated ... tourniquet was placed ro nd the leg in the distal part of the tibia to stop blood flow to the foot ... main vein draining the foot was cut open on the medial side of the tarsus–met tarsus. The main foot ... was cannulated and perfused at constant pressure of 40 mmHg w th solution similar t that used ... perfusion of completely isolated feet, wh le the perfusate escaped through the opened ein. Change ... perfusat flow d ring cooling a d rewarming of the foot was measured w th an electromagnetic flow ... ducer placed on the inflow side of the perfusion system. The experiment was performed in the temper... range between 25°C and 5°C.

Results

Perfusion of isolated foot During perfusion at constant flow with both the foot and ... perfusion fluid at 18–25°C, electrical stimulation of the nerve trunks running alongside ...

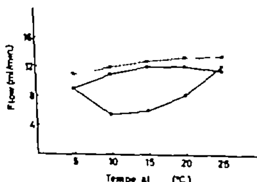


...t of intravenous injection of adrenaline (5 µg) on mean aortic blood pressure, mean blood ...the digital artery (left foot, in ice-water), subcutaneous digital temperature of left foot, and ...s digital temperature of right foot (in air 22–23°C)

Discussion

...r provides evidence that in the ice-water immersed feet of ducks the blood flow ...he web is mainly governed by the digital arterial blood pressure. It is moreover ...that rather steep pressure gradients and accordingly high vascular resistance exist ...arterial outflow vessels to the extremity

...nstrictor response of the vascular smooth muscle in the foot to both exogenous ...line and nerve stimulation was shown to subside progressively as the foot tissues ...d on ice-water immersion. Responses to noradrenaline can be elicited even at ...t fairly high dose levels are required. Normal levels of adrenaline and noradrenaline



...Changes in perfusate flow during ...) and rewarming (O) of perfused ... with intact nerve supply. Dotted ...shows temperature dependent flow ... similarly perfused completely ...out (Millard and Reite 1975). In the ...mparison the flow change pattern ...rewarming is reversal of that ob...ring cooling.

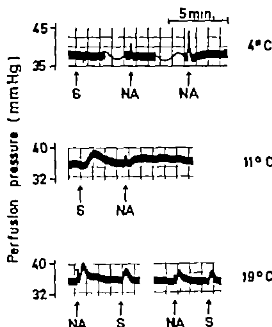
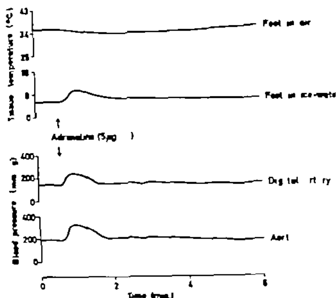


Fig. 2. Influence of temperature on the vasc. response to electrical stimulation of artery (S) to injection of noradrenaline (NA) in the perfused foot of the duck At 4°C there was [illegible] detectable effect of stimulation of the artery [illegible] low dose of noradrenaline (0 02 µg) also [illegible] no effect, whereas a higher dose (0 1 µg) [illegible] a weak but longlasting rise in perfusion pressure [illegible] 11°C periarterial nerve stimulation (S) had a [illegible] effect, and the effect of a low dose of noradre[illegible] (0.02 µg) was fairly strong and longlasting At [illegible] noradrenaline (NA) produced marked effects at [illegible] levels of both 0 01 and 0 02 µg. The effect of [illegible] arterial nerve stimulation (S) was of the same [illegible] tude as that of the lowest dose of noradrenal[illegible]

stayed 40–50 mmHg lower Also with one foot immersed in ice-water and the other [illegible] the blood pressures in the digital arteries of the two feet remained within the same [illegible]

Effect of drugs on blood pressure and foot temperatures Adrenaline and noradrenaline doses of 2–10 µg of the base given i v produced a marked rise in blood pressure both in t[illegible] aorta and in the digital arteries The pressure rise was accompanied by a rapid rise of [illegible] 3 to 15 C in the subcutaneous digital temperature in the ice water immersed foot while [illegible] opposite response of a decrease of 1 to 3 C or no detectable change in digital tempera[illegible] characterized the warm foot (Fig 3) As the blood pressure fell to the level prevailing be[illegible] injection of adrenaline and noradrenaline the temperatures of the two feet gradually retur[illegible] to their previous ranges The effects of adrenaline and noradrenaline on blood pressure [illegible] foot temperatures remained after both cervical vagal trunks were severed, and they [illegible] persisted after administration of atropine (3 mg i v) and the β-adrenergic blocking ag[illegible] propranolol (3 mg i v) After administration of the α-adrenergic blocking agents phent[illegible] mine (6 mg i v) or phenoxybenzamine (6 mg i.v) both the blood pressure effects of adre[illegible] line and noradrenaline and their effects on foot temperature were reduced The α-adrener[illegible] agents *per se* caused a foot temperature rise of up to 4–5 C when the initial temperat[illegible] were below 4 C and 30°C in the ice water immersed foot and the foot in air respectiv[illegible] At higher foot temperature levels the rise was less marked or absent

Perfusion of foot with intact nerve supply Cooling of duck feet perfused at constant pres[illegible] with the nerve supply intact showed a rapid decrease in flow from 25°C down to about 15[illegible] Between 15°C and 10°C the flow decreased more slowly and from 10°C down to 5 C [illegible] flow increased. Rewarming from 5°C caused a further increase in flow until the tempera[illegible] reached 15°C. From 15°C the flow decreased and at 25°C it stabilized slightly below [illegible] initial level Changes in perfusate flow during cooling and rewarming as recorded in on[illegible] the experiments are shown in Fig. 4

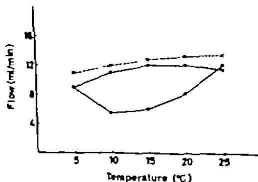


3. Effect of intravenous injection of adrenaline (5 µg) on mean aortic blood pressure, mean blood ... in the digital artery (left foot, in ice-water), subcutaneous digital temperature of left foot, and ... digital temperature of right foot (in air 22–23°C)

Discussion

... study provides evidence that in the ice-water immersed feet of ducks the blood flow ...ough the web is mainly governed by the digital arterial blood pressure. It is moreover ...arent that rather steep pressure gradients and accordingly high vascular resistance exist ...ng the arterial outflow vessels to the extremity

The constrictor response of the vascular smooth muscle in the foot to both exogenous ...radrenaline and nerve stimulation was shown to subside progressively as the foot tissues ... cooled on ice-water immersion. Responses to noradrenaline can be elicited even at ...5°C, but fairly high dose levels are required. Normal levels of adrenaline and noradrenaline



...g. 4. Changes in perfusate flow during ...oling (●) and rewarming (○) of perfused ...ck foot with intact nerve supply. Dotted ...e ( ) shows temperature dependent flow ...anges in a similarly perfused completely ...rvated foot (Mallard and Rasts 1975). In the ...ter preparation the flow change pattern ...ring rewarming was reversal of that ob...rved during cooling

in circulating blood are *probably too low* to exert a constrictor effect on the blood vessels of the foot at tissue temperatures lower than 8–10°C.

In the completely isolated duck foot perfused at constant flow cooling from 25 to 5 caused an increase in perfusion pressure of about 10 per cent, indicating a resistance increase of similar order of magnitude. Since the viscosity of water is about 70 per cent higher at 5°C than at 25°C (Weast 1973) the hindrance must be reduced by about 35 per cent. The reduction in hindrance at 5 C, as calculated from Poiseuille's equation, can be ascribed to cold-induced vasodilatation

Flow changes seen during cooling and rewarming of the partly isolated perfused duck foot preparation were different from the flow changes previously observed in similarly perfused completely isolated feet (Millard and Reite 1975) The difference can be explained by the disappearance *and reappearance* of sympathetic vasoconstrictor tone in feet with intact *nerve supply* Sympathetic nervous control seemed to be absent or very weak at temperatures below 8 C.

According to the present results any stimulus increasing the levels of adrenaline or noradrenaline in the blood or causing a general increase in sympathetic vasoconstrictor nerve activity will produce vasoconstriction mainly if not only in the warmer tissues. The resulting rise in *blood* pressure will cause a blood flow increase in the distal part of the water immersed foot This mechanism for local blood flow increase is not operable in tissues where vasoconstriction will affect the resistance along the entire foot vasculature. The main influence of sympathetic nerves on the blood flow through ice-water immersed feet must be exerted on the larger arteries in the tarsal–metatarsal region where the temperature is higher than in the web Maintenance of a blood supply adequate to prevent cold injury to the feet of ducks is *therefore* seemingly not due to humoral or neurohumoral mechanisms *operating in the coldest tissues*. Cold *per se* produces dilation of the blood vessels *in the foot of the duck* and the vessels remain dilated because they are unresponsive to sympathetic nerve stimulation and physiological levels of adrenaline and noradrenaline in circulating blood The usefulness of controlling blood flow through cold-exposed tissue without depending on local control mechanisms is obvious. A locally induced vasoconstriction at low temperatures would be so long-lasting that damage would almost certainly follow. The only alternative would be to have an additional *local vasodilating mechanism*. Our data give no information on the relative *contribution* of nutritional blood vessels and arteriovenous *anastomoses in the* described responses. Fluctuations in the digital temperature may represent changes in total flow to the foot or redistribution of blood flow within the foot.

The interpretation of our results as given above is compatible with previous reports. Folkow Fuxe and Sonnenschein (1966) showed that blood vessels supplying skeletal muscle in the duck have a more dense sympathetic innervation and show a greater drop in pressure along the larger arteries than what is commonly found in mammals. If there is cold blockade of vasoconstrictor nerves in the peripheral small arteries and arterioles *in a mammal*, part of the vascular resistance will disappear which in turn *may give rise to a pronounced* local increase in blood flow Consequently the tissue *temperature will again rise* the cold blockade will be abolished, *and the resistance reappear* This pattern may partly explain the "hunting" reaction first described by Lewis (1930) In the duck consider [illegible]resistance to [illegible]

...s in the large arteries after cold blockade of the constrictor nerves supplying the more ...pheral smaller ones. The typical hunting reaction which did not appear during our ...s., should, accordingly be absent or markedly reduced. Fluctuations in subcutaneous ...al tissue temperature as demonstrated in the present work were small and irregular ... both the cold and the warm foot were affected. Evidence has been presented that the ...nting reaction is also absent in the feet of some arctic mammals (Henshaw Underwood Casey 1972), and that it is reduced in the hands of man acclimatized to cold (Krog *et al* ...).

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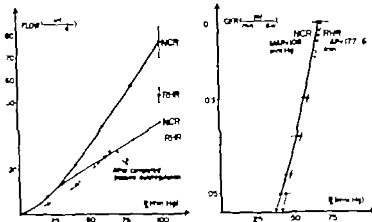
in circulating blood are probably too low to exert a constrictor effect on the blood vess the foot at tissue temperatures lower than 8–10°C.

In the completely isolated duck foot perfused at constant flow cooling from 25 to 5 caused an increase in perfusion pressure of about 10 per cent, indicating a resistance crease of similar order of magnitude Since the viscosity of water is about 70 per cent h at 5 C than at 25°C (Weast 1973) the hindrance must be reduced by about 35 per cent T reduction in hindrance at 5 C, as calculated from Poiseuille s equation, can be ascribed cold-induced vasodilatation

Flow changes seen during cooling and rewarming of the partly isolated perfused duck preparation were different from the flow changes previously observed in similarly perf completely isolated feet (Millard and Reite 1975) The difference can be explained by disappearance and reappearance of sympathetic vasoconstrictor tone in feet with nerve supply Sympathetic nervous control seemed to be absent or very weak at temper below 8°C

According to the present results any stimulus increasing the levels of adrenaline r noradrenaline in the blood or causing a general increase in sympathetic vasoconstr nerve activity will produce vasoconstriction mainly if not only in the warmer tissues T resulting rise in blood pressure will cause a blood flow increase in the distal part of the water immersed foot. This mechanism for local blood flow increase is not operable in tissues where vasoconstriction will affect the resistance along the entire foot vascular The main influence of sympathetic nerves on the blood flow through ice-water immers feet must be exerted on the larger arteries in the tarsal–metatarsal region where the tempe ture is higher than in the web. Maintenance of a blood supply adequate to prevent c injury to the feet of ducks is therefore seemingly not due to humoral or neurohumoral me anisms operating in the coldest tissues. Cold *per se* produces dilation of the blood ves in the foot of the duck and the vessels remain dilated because they are unresponsive sympathetic nerve stimulation and physiological levels of adrenaline and noradrenaline circulating blood The usefulness of controlling blood flow through cold-exposed tis without depending on local control mechanisms is obvious. A locally induced vasoconst tion at low temperatures would be so long-lasting that damage would almost certainly fol The only alternative would be to have an additional local vasodilating mechanism. Our d give no information on the relative contribution of nutritional blood vessels and arte venous anastomoses in the described responses. Fluctuations in the digital temperature represent changes in total flow to the foot or redistribution of blood flow within the f

The interpretation of our results as given above is compatible with previous repo Folkow Fuxe and Sonnenschein (1966) showed that blood vessels supplying skeletal mu in the duck have a more dense sympathetic innervation and show a greater drop in pres along the larger arteries than what is commonly found in mammals. If there is cold block of vasoconstrictor nerves in the peripheral small arteries and arterioles in a mammal, of the vascular resistance will disappear which in turn may give rise to a pronounced increase in blood flow Consequently the tissue temperature will again rise, the cold block will be abolished and the resistance reappear This pattern may partly explain the hun ... first described by Lewis (1930) In the duck considerable resistance to flow



Left part shows the average relationship ($\pm$S E.) between arterial pressure (P_A) and flow (per g dry weight) in 9 pairs of maximally vasodilated NCR and RHR kidneys, both before and after the establishment of "passive" autoregulation (see Methods). For each P_A level renal flow resistance per unit weight is clearly increased in the RHR kidney which during 4–5 weeks had been exposed to an increased MAP.
Right part shows the average relationship ($\pm$S E) between P_A (after completed autoregulation) and glomerular filtration rate (GFR; ml/min g dry tissue weight) for 9 pairs of NCR and RHR kidneys. Note the relationship, which reflects the structurally determined pre- to postglomerular resistance ratio, is significantly shifted to the right in RHR, in striking contrast to the situation in SHR with established hypertension (Folkow *et al* 1977). — Correction for passive autoregulation (*cf* left part) would make both curves much steeper but with little influence on their mutual relationships.

Hypertension was induced in $2\frac{1}{2}$ month old NCR by clipping of the left renal artery leaving the right kidney intact (two-kidney RHR). After 4–5 weeks, when mean arterial pressure (MAP) was 177 ± 6 mmHg in these RHR, acute paired experiments were performed on the isolated right RHR kidney in comparison with those from matched NCR, where MAP was 108 ± 3 mmHg.

RHR and NCR right kidneys were decapsulated and perfused with plasma substitute (Dextran solution) during maximal dilatation and at room temperature to minimize tubular reabsorption (Folkow *et al* 1977). Urine formation was continuously measured by sensitive weighing devices and GFR was determined spectrophotometrically from the concentrations of Cr-EDTA in perfusate and urine. The relationships between P_A and GFR were determined by recordings of urine flow at various P_A, starting where urine flow was just initiated. Then the GFR values were calculated, revealing in this setup reabsorption around 60 per cent, equal in both kidneys. The P_A–GFR relationships were then plotted at the P_A levels after established passive autoregulation, representing the sum of the "true" pressure and the tissue pressure rise due to increasing nephron distension. Net filtration is enhanced at higher glomerular capillary pressures. Subsequently the pressure–perfusate flow relationships were determined, before (P_A taken exactly 5 after the prompt flow shift) and after the full establishment of passive autoregulation. — Finally the dry weights of the kidneys were determined so that possible differences in size should not affect calculations of GFR and flow per unit weight.

Fig. 1, left part, shows the relationships between pressure (P_A) and perfusate flow/min g kidney weight in RHR and NCR, both before and after established passive autoregulation during maximal vasodilatation. Obviously total renal vascular resistance per unit tissue weight was considerably higher in the pressure-exposed right RHR kidney compared with the NCR one. However the intact RHR kidney had also increased its mass by hypertrophy

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From the Department of Physiology University of Göteborg, Sweden

Structural Renal Vascular Changes in Renal Hypertensive Rats (RHR)

By

B. Folkow G Göthberg S Lundin and S E. Ricksten

Recently (Folkow *et al* 1977) total renal vascular resistance as well as the ratio bet the pre and postglomerular resistances were compared in paired perfusions of max vasodilated kidneys from spontaneously hypertensive rats (SHR) and matched normoten controls (NCR) The comparison was performed both in 5 week old animals when SHR in the phase of borderline hypertension" and at 3–4 months of age when the SHR hyp tension is fully established Differences in the structurally determined pre to postglomer resistance ratio were estimated by comparing the respective relationships between perfusion pressure (P_A) and glomerular filtration rate (GFR) If this ratio is increased one of the animals, the corresponding P_A–GFR curve becomes parallelly displaced to right along the P_A abscissa. If the glomerular filtration capacity is reduced as well steepness of the curve is correspondingly depressed This technique has much in com with comparisons of the pre to postcapillary resistance ratio and the capillary filter capacity in other systemic circuits (*cf* Folkow *et al* 1974).

In the very young animals the SHR–NCR curves relating P_A to GFR were almost iden with the SHR curve just barely displaced towards the right In contrast, in the 3-4 mo old animals the SHR curve was displaced as much as 30–40 mmHg towards higher levels compared with the NCR one, but the curve steepness was still largely equal.

These results strongly suggest that the "triggering" neurohormonal pressor influences SHR rapidly induce a structurally based vascular narrowing which in the kidneys is con largely to the *preglomerular* vessels, responsible also of the wellknown renal blood f autoregulation In other systemic circuits this process of structural autoregulation involv the entire precapillary resistance compartment (Folkow *et al* 1974 1977). The postglomer resistance appeared to be, if anything, slightly reduced in SHR compared with NCR.

This rapid structural increase of the pre to postglomerular resistance ratio in SH implies a most efficient resetting towards hypertensive levels of the renal "long-term baroc function" (*cf* Guyton *et al* 1974), but so far (up to 4 months of age) leaving the glomer filtration capacity largely unchanged. The process is of the same principal nature as the resetting systemic precapillary resistance, the "short term" barostats and the left ventr (*cf* Folkow Hallbäck and Noresson 1977). The question arises whether exactly simil structural changes of the renal vascular bed occur in all types of hypertension. A compar ison was therefore performed on kidneys from rats with renal hypertension (RHR) and NC

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From the Institute of Physiology University of Bergen, Norway

Effect of Dehydration on Renal Blood Flow in Dog

By

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Abstract

, and I. Tyssebotn. *Effect of dehydration on renal blood flow in dog.* Acta physiol. 1977 *101* 257–263

dehydration on intrarenal blood flow was investigated in 11 dogs, using polarographic of H_2-gas desaturation for measuring local blood flow in inner cortex (ICF) and outer . Dehydration was induced by 48 h water deprivation +2 300 mg ethacrynic acid (EA) per efore the experiment. Compared to control group (n = 9) ICF was markedly reduced to /min g (control 3.23 ± 0.64) whereas OCF 3.29 ± 0.80 ml/min g was nearly unchanged ± 0.85). The ratio OCF/ICF was increased to 1.37 (1.11). Further dehydration by hypertonic lysis for 3 h increased Hct to 60 ± 4 and further reduced OCF and ICF without significant e OCF/ICF-ratio. At Hct above 55 sudden and intermittent changes in local cortical blood corded randomly at individual electrode sites, showing ischemic periods lasting for 1 to flow changes were observed in 13 of 14 expts and were not accompanied by changes in RBF led that moderate dehydration causes greater reduction of ICF than of OCF. Severe de- ves in addition rise to patchy intermittent ischemia in both cortical layers.

nt types of dehydration, ranging from severe salt to pure water loss, will in time decreased plasma volume, leading to reduced cardiac output, arterial pressure, d flow and glomerular filtration rate. Dehydration and hypovolemia predispose oliguric renal failure and one might suspect that changes of intrarenal distribution flow might be involved in addition to the reduction of local blood flow — While enal flow distribution has been extensively studied in other low flow states such as ggic hypotension and infusion of various vasoconstrictors, we are not aware of any ies in dehydration, except for the preliminary report by us (Kirkebø and Tyssebotn easuring a greater reduction in inner than in outer cortical blood flow with periods r cortical ischemia.

ration is most easily induced by a saluretic, and we have used ethacrynic acid ich gives a shortlasting, but very high plasmaisotonic diuresis.

the purpose of our study was to investigate RBF and the local cortical blood tribution with the hydrogen washout method during different stages of dehydration d by a combination of water deprivation, EA and hypertonic peritoneal dialysis.

(about 45 per cent) and the characteristic structural cardiovascular changes noted i l by Lundgren (1974) were also observed in the present RHR.

The right part of Fig. 1 shows the P_A–GFR relationships which are almost identi RHR and NCR despite the fact that the RHR hypertension had lasted long enough weeks) to fully complete the hypertrophic structural adaptation of heart and vessels. T nearly congruent RHR–NCR curves are in sharp contrast to the marked right-hand dis ment of the P_A–GFR curve in SHR with established hypertension (Folkow et al. 197

The question arises why this is so. SHR hypertension seems to be initiated mainly neurogenic mechanisms which predominantly affect the preglomerular vessels thus anything, lowering pressure at and beyond the glomerular level. However in RH increased formation of angiotensin II seems mainly responsible of the pressure inc and in the kidneys this substance preferentially constricts the postglomerular arter (cf Leyssac 1976) Consequently in the pressure-exposed RHR kidney transmural c cular pressure is raised also in the postglomerular vessels. Since the local pressure i main determinant of structural cardiovascular adaptation, both sets of renal res vessels are likely to be structurally altered in RHR, and largely to a similar extent T while total renal resistance to flow per unit tissue weight is increased in the intact R kidney there is hardly any alteration of the pre to postglomerular resistance ratio, in contrast to the increased ratio in established SHR hypertension, at least as long as t vascular wall hypertrophy is involved. This implies that the structural resetting of the re barostat function may be quite differently organized in SHR and RHR, and in a w which helps to explain why hypertension is more readily reversed in RHR than in SHR Lundgren 1974 Weiss 1974) From the largely unchanged pre to postglomerular resi ratio in RHR it follows that a MAP reduction does here *not* imply any undue lowering glomerular filtration pressure, in contrast to established SHR hypertension where a m normalization of MAP may even eliminate GFR

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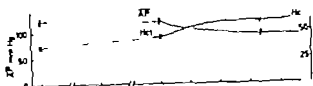
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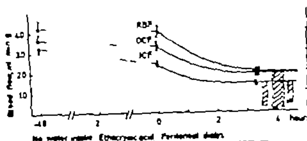
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...and blood flow (± S.E.), ...t in control dogs (9), ...dogs (14) exposed to ...rivation and ethacrynic ...id 4 h before measure- ...d thereafter to hypertonic ...l dialysis. Hatched bars ...ally indicate periods with ...occurrence of patchy ...t blood flow.



...creased to 45 5. $\overline{AP}$ was 120±10 mmHg (range 100–135 mmHg). RBF and OCF ...ined almost at the same level as in the control group presented above (Fig. 1). ICF ...other hand was reduced to 2.40±0.47 ml/min g, significantly lower (p 0.001) ...OCF (3.29 0.80 ml/min g) measured simultaneously. The ratio OCF/ICF had in-...d to 1.37. The urine flow was low and urine osmolarity about 1 000 mosm/l at this ...of dehydration, showing that the effect of EA had passed. The hydrogen washout ...were monoexponential, without any sudden changes in washout rate at individual ...ode sites.

...re dehydration. The expts. were continued with repeated peritoneal dialysis using a ...tonic glucose Ringer solution. Urine flow ceased (below 0.01 ml/min) at Hct bout ...fter 3½ h of dialysis an extra fluid loss of approximately 0.5 l had been obtained and ...ad increased to 60±4. $\overline{AP}$ fell to 95±14 mmHg, while RBF decreased to 1.95±0.70 ...m g, showing a rise in vascular resistance of bout 60% (Fig. 1), including the rise ...ood viscosity.

...th OCF and ICF were reduced to the same extent, i.e. about 60% of the local flow ...ned by moderate dehydration, thus maintaining the high OCF/ICF ratio of 1.35 ...n this severely dehydrated state.

...t Hct higher than 55 shifts in local blood flow could be recorded as sudden changes in ...out rate on the otherwise monoexponential hydrogen clearance curves. The flow could ...r from near control blood flow to zero flow at the same electrode site at consecutive ...learance periods or at different time in the same washout period. Fig. shows an example ...re the H washout begins at a rate of 1.98 ml/min g, falls to 0.37 for about 1 min, ...to 1.20 ml/min g for another minute, falls to zero for 3½ min and again increases to ...first washout rate of about ml/min g. In this single hydrogen washout curve 5 different ...rance rates were recorded, and the semilogarithmic plot shown in the middle panel is ...ar at all different flow rates indicating abrupt changes in the local flow. As the zero line ...was ery stable, flow could be determined down to very low level of H concentration. ...show in Fig. 3 from 1 expt. the washout rat changed independently at different electrode

Methods

The investigation comprised one series of experiments on dehydration induced by EA and pe dialysis. An additional series of 9 expts. performed simultaneously with the same technique ... (Tyssebotn and Kirkebo 1975).

Before surgery the dogs were anesthetized by pentobarbital, 25 mg/kg b t and additional were given when needed The dogs were intubated with a rubber endotracheal tube and breathed taneously through the whole experiment. Polyethylen catheters were placed in the saphenous ... mean arterial pressure ($\overline{AP}$) recording and in the saphenous vein for infusions. The kidney was ... retroperitoneally through a flank incision, nd the renal artery gently dissected free for measure the total renal blood flow (RBF) by a Nycotron electromagnetic flowmeter The flow meter was ... directly on a femoral artery A polyvinyl catheter was inserted into the renal artery (Herd and ... with the tip directed upstream, for infusion of the hydrogen saturated isotonic saline The ... cannulated by a silicone rubber catheter for urine flow measurements.

3 L shaped platinum electrodes were placed in outer half of the cortex with the ... tips 1–4 mm deep and 3 electrodes in the inner half of the cortex 5–8 mm deep. The ... electrodes were fixed to the renal capsule by two sutures and carefully covered by perirenal fat. The ... was closed by towel clips before measurements started. Hydrogen gas concentration around the ... electrode tip was determined polarographically at a polarization potential of +0.2 V ... electrode placed subcutaneously on the hip (Aukland *et al* 1973).

The amplified electrode current was recorded on a 6-channel recorder (Rikadenki Kogyo Co. ... B-64) For recording of a washout curve, 1–4 ml hydrogen saturated saline at 37°C ... the renal artery until constant H_2-concentration was obtained at the different electrodes ... course of ½–1 min. The i jection was then suddenly stopped and the washout curves ... thereafter plotted on a semilogarithmic paper Blood flow in ml/min per g tissue ... th formula 0.693/T½ where T½ is the half life in mi utes of the hydrogen concentration ... Urine osmolarity was determined by freezing point osmometer (Advanced Instr.).

The kidney was excised at the end of the expt., drained and weighed, and the location of each ... tip was carefully examined

Dehydration by water deprivation EA and peritoneal dialysis The effect of dehydration on renal flow was determined in 14 expts on 11 dogs (of both sexes) weighing 14–24 kg The dogs were deprived for 48 h before the experimental measurements started. 4 h before the expt. 2 ... given per os. This produced a great plasma isotonic diuresis. The dogs had free access to dried ... ntil 18 h before surgery

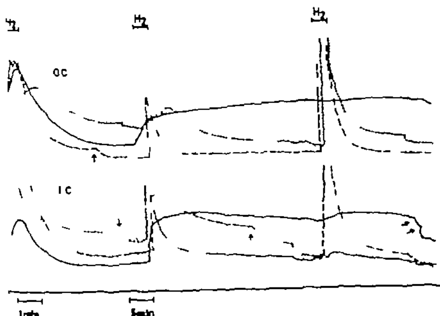
A silicone rubber catheter 6–7 mm diameter with ma y side holes was placed in between the ... for infusion of hypertonic dialysing fluid consisting of 7 glucose in Ringer's solution ... 37 38°C. A small plastic cup with side holes was put between the intestines as a reservoir to facilitate removal of the dialysate.

At intervals of 30 min, 3–500 ml dialysate was infused and withdrawn after 20 min using a total of 1 500–2 000 ml dialysate. The hematocrit (Hct) was checked every 20 ml nd the rise in Hct ... indicate the degree of hydration. Hydrogen washout curves were determined each 15 ... period of increasi g dehydration. The peritoneal dialysi was continued until Hct had reached ... Thereafter hydrogen clearance was measured every 5–10 min for 1 2 h. T-tests ere used for ... evaluation.

Results

Control measurements In a previously published study performed simultaneously and the same technique in this laboratory (Tyssebotn and Kirkebo 1975), OCF and ICF ... dogs were 3 59 ± 0 85 ml/min g and 3.23 ± 0.64 ml/min g (mean ± 1 S D) respectively showing a ratio OCF/ICF of 1 11 RBF was 4.20 ml/min g drained kidney weight mean Hct was 37 and average $\overline{AP}$ was 122 mmHg in these dogs.

Dehydration by water deprivation and EA 48 h of water deprivation and 24 h after a ... of 2–300 mg EA given per os, the body weight was reduced by ab ... and the average



H₂ washout curves of 3 single electrodes in outer (OC) and 3 in inner (IC) cortex of a dog dehydrated ...neal dialysis for about 4 h, showing sudden changes in local blood flow. See text.

...other. In some expts., however, the ischemic episodes were confined to only one ...ode and were short-lasting, as further dehydration generally lead to lower flow and ...pearance of the intermittent changes.

Discussion

...ts of dehydration. To study the effect of dehydration it would have been desirable to ...sure local cortical blood flow repeatedly from chronically implanted electrodes. How... previous measurements have shown that the recorded local renal blood flow fell ...kedly the first days after chronic implantation of platinum electrodes, while RBF was ...tained (Kirkebø and Tyssebotn 1974). Therefore measurements with chronically ...lanted electrodes have not been performed on the same dogs before and after longlasting ...er deprivation. As control for this series of expts. are used measurements done simul...neously with the same technique on 9 separate dogs (Tyssebotn and Kirkebø 1975). The ... values from the chosen control material are slightly higher than control flow obtained ...n 5 dogs used for the study of the acute effect of EA injection, but the ratios OCF/ICF ...e identical. Both series agree well with control flow previously obtained by the H_2 ...hout technique (Løyning 1971, Aukland *et al.* 1973, Kirkebø and Tyssebotn 1974).

...n dogs dehydrated about 9% of b wt. by water deprivation and a dose of EA given the day ...ore measurements, RBF and OCF were only insignificantly lower than in the control ...up, even though Hct had increased from 37 to 45. ICF fell to 74% of control, giving a rise ...the ratio OCF/ICF from 1.11 to 1.37. The high urine osmolarity showed that the effect

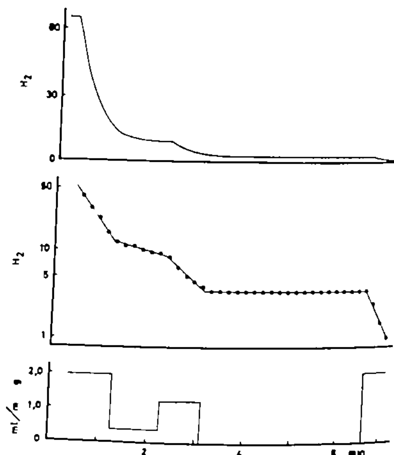


Fig. 2. H_2 washout curv of a single electrode (above) H replotted on semilogarithmic paper panel) and the derived blood flow values (below), showing sudden, intermittent changes in loc See text.

representing different zones. During the 3 washout periods presented in Fig. 3 at k sudden increases in clearance rate (arrows) are observed on different electrodes in zones. Fig. 3 also shows zero or very low clearance in 5 more curves indicating zero at 2 electrode sites in the middle part and 3 in the right part of the panel When in conse washout periods curves showed change from high to very low clearance rate a sudde gradual elevation of base line during the first washout period frequently could be us detect the actual moment when the abrupt reduction in blood flow occurred.

Generally more than half of the electrodes showed marked shifts in H washout appearing at different electrode sites at different time, and the distribution of such ch occurred randomly in the outer and inner cortex. No syncronisations of the flow chang different electrode sites was detected. Sudden changes in RBF was rarely recognized dicating that the observed changes in local cortical flow took place in relatively small reg probably not exceeding 10 % of the total cortical volume The duration of the isch periods varied greatly and unpredictably from less than 1 min up to 1 h However since method does not permit continuous measurements, the beginning and end of the isch was often undetermined. Such patchy intermittent ischemia was observed at least at electrode site in 13 out of 14 expts. and in some expts. involved all ode sites at one

...striction is involved for instance at the level of the arcuate or interlobular arteries. ...lmentary expts. we have not been able to prevent the phenomenon by heparinization ...ha receptor blocade. However the variability and the fact that further dehydration ...ally lead to lower flow and disappearance of the intermittent changes have complicated ...ematic search for the mechanisms involved.

...are aware of only one previous report on flow changes comparable to those described ... In patients with hepatic cirrhosis and greatly reduced renal function Epstein et al. ...) observed sudden changes in externally monitored ^{133}Xe washout curves. However ... the published results it seems likely that the intermittent flow reduction in those cases ...ved the whole or greater parts of the cortex, whereas only small cortical regions seemed ... involved in the present expts.

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of EA had passed and that the preferential reduction in ICF was due to an effect of dehy-tion. It should be noticed that Hct is lowered by bleeding and increased during dehydr and may be the key factor to explain why the ratio OCF/ICF is raised during dehydrat and not during hemorrhage. Nybo Rasmussen (1976) has reported that in hydrop inner medulla was perfused with red cell poor blood. At the high Hct obtained by dehydr tion the red cell separation in preceding vessels might be less pronounced, so that the viscosity increased more in juxtamedullary than in outer cortical zones.

Severe dehydration induced by hypertonic peritoneal dialysis reduced both OCF an ICF to 60 % of blood flow at moderate dehydration, and thus maintained a high ratio OCF ICF The flow reduction must be caused mainly by an increase in blood viscosity as Ht rised to 60 thereby increasing total renal vascular resistance. Possibly the increase in vi cosity (at least 1 5 times) (Chien *et al* 1966) even covers that a slight vascular dilation took place. Since MacDonald (1976) reported that an increase of Hct caused no change in RBF this suggests that a vascular dilation has compensated the increased viscosity Theoretical decreased $\overline{AP}$ and blood volume should induce a high concentration of vasoconstrictor at least of ADH noradrenaline and angiotensin therefore it is remarkable that a noticeab vasoconstriction does not appear

Intermittent and patchy flow reduction In all previous work by the H washout metho we have observed essentially monoexponential washout curves and a relatively homogeneo blood flow distribution within the renal cortex. However in states of severe dehydration Hct above 55 or during hind limb tourniquet shock shifts in local flow occurred sudden on single electrode sites without detectable changes in RBF The records showed irregul patterns of intermittent and patchy reductions of blood flow ranging from control to ze flow and from less than 1 min up to 1 h There is no indication of electrode movement explanation for the abrupt changes in the washout rate, since a movement would induce electrical spike *i.e* an abrupt elevation of the washout curve which within 1 min would back to a normal washout. Such spikes are never observed in connection to the abru shifts in washout rate On the other hand, even if electrodes moved and detected a n washout rate it would just be another indication of areas in cortex with different wash rates.

Most of the washout rates are monoexponential down to less than 10 saturation, the unlinear end of the desaturation curve depend on the change in zero line. In our stu the zero line occasionally are observed to increase at low flow rates, but this elevation small compared to changes induced by the hydrogen concentration. Also, our studies hemorrhagic hypotension and vasoconstrictor infusions (Aukland *et al* 1973 Kirke and Tyssebotn 1974 Tyssebotn and Kirkebo 1975) have never shown any abrupt changes flow rate, even when RBF was very low (down to 15% of control flow) which furthermo suggest that the sudden changes in washout rate are not due to hyperreactivity only of tissue immediately around the electrode tip.

Theoretically the electrodes use H continuously but as shown by the minimal desatu tion rate in Fig. 3 and by Tonder and Aukland (1974), the half life of H current in a stric limited cavity (dental pulps), was more than 1½ h after circulatory arrest

The reversibility and the rapidity of the changes in local corti dica

le the splanchnic vascular bed is the prime locus of action of endotoxins in dogs, the monary vascular bed constitutes the main target conduit in cats (Gilbert 1960 Kuida et al. 1961 Liebel et al. 1964).

Material and Methods

Experiments were conducted on 9 cats of both sexes and weighing from 1.6 to 3.3 kg. The cats were anesthetized with chloralose (100 mg/kg). They were intubated and ventilated with volume controlled respirator. The acid-base and blood gas state was determined by the Astrup technique, and corrections to physiological limits were made by adjusting the pulmonary ventilation or by giving concentrated sodium bicarbonate intravenously during the preparative phase. The oesophageal temperature was kept 36-38°C during the preparatory phase.

Polyethylene tubings were inserted into the thoracic aorta and the inferior caval vein via the femoral vessels. A left thoracotomy was performed, and sympathetic efferent postganglionic activity was recorded from fibres of the 3rd cardiac nerve. The fibre to be recorded was cut distally to the electrodes. The splenic nerve activity was recorded from the cranial ends of thin nerve fibres in the pedicle of the spleen reached through a left upper laparotomy. The aortic arch baroreceptor discharges were recorded on the left from the left vago-sympathetic trunk.

Endotoxin Escherichia coli was given intravenously (1 mg/kg) within 10 s as saline suspension (1 ml/kg). Recordings on the magnetic tape were started 1-2 min before the endotoxin injection and were carried continuously up to 5 min 20 s. Thereafter recordings, lasting 20 s, were made starting at 9 and 15 min. The number of nerve impulses per second was calculated with a multichannel analyser (Nokia LP 4840, Nokia Electronics, Finland). The means of the relative changes of discharge rates were calculated in all recordings of the same fibre type for each 20-s period before and after the endotoxin injection. The means of absolute changes were calculated for the heart rate and the aortic and central venous pressures. The significance of changes was calculated with the Student's t-test.

For more detailed description of the methods see Halinen (1976)

Results

The average reactions of the parameters recorded are illustrated in Fig. 1 and Fig. 2 exemplifies a set of recordings from an individual expt.

During the control period, the mean heart rate (±S.D. Fig. 1) was 181 ±33 per minute. The systolic aortic pressure was 120±38 mmHg and the diastolic one 94±32 mmHg.

Following the endotoxin injection, the mean heart rate immediately decreased slightly (Fig. 1). It then increased to attain its maximum at about 3 min (220±30 per minute). Subsequently following a bradycardic transient, the mean heart rate decreased to control level.

The mean changes in the systolic and diastolic pressures were somewhat opposite to those in the heart rate. The lowest pressures, the systolic 75 mmHg and the diastolic 45 mmHg, coincided with the bradycardic transient (Fig. 1). Individually (Fig. 2), there was often, following the initial pressure drop, one or two bouts of relative hypertension also discernible in the curves of mean changes (Fig. 1). At the end of the experimental period, hypotension still prevailed (Fig. 1 and 2).

The mean aortic baroreceptor afferentation (Fig. 1) decreased to 30 per cent of the control level. In some cats it ceased during the nadir of the aortic pressure (Fig. 2).

The cardiac sympathetic efferent discharges increased at 3 min after the injection but diminished during the rising aortic pressure after its first fall. During the ensuing secondary bout of hypotension, the cardiac sympathetic efferentation increased up to 1.40 times (Fig. 1).

Acta physiol scand. 1977 101 264-269

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Circulatory Reflex Responses during the Initial Stage of Feli
Endotoxin Shock

By

M O HALINEN M O K. HAKUMÄKI and H S. S. SARAJAS

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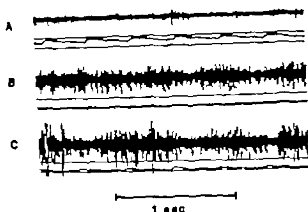
Abstract

HALINEN, M O M O K HAKUMÄKI and H S S. SARAJAS. *Circulatory reflex r during the initial stage of feline endotoxin shock* Acta physiol. scand. 1977 101 2

Cardiovascular and a tonomic nervous responses to an injection of *E. coli* endotoxin were fol up to 15 min. in cats anesthetized w th chloralose and given artificial respiration. Within 60 endotoxin induced drop of aortic pressure, with simultaneous cardiac acceleration and sligh venous hypertension. There was an associated almost complete cessation of the aortic arch bar afferentation. The cardiac ympathetic efferentation increased up to 1 4 times the control le maximum. The splenic sympathetic efferentation increased up to 10.6 times the control level at t the 15 min period when the other parameters studied showed a trend to control level. The sy autonomic system seems to be activated through cardiovascular receptors sensing hemodynami touched off by endotoxin-induced release f vasoactive substances.

Key ords. Endotoxin, autonomic nervous system, cardiac sympathetic efferent discharge sympathetic efferent discharges, baroreceptor discharges

The release of vasoactive agents is likely to be the main cause of hypotension an induced by endotoxin (Gilbert 1960 Hinshaw 1971). On the other hand, endotox been proposed to be neurotoxic agents (Nagler and Levenson 1971) activating t pathetic nervous system directly (Lillehei *et al* 1964). From the behaviour of the catecholamines and heart rate, in turn it has been concluded that endotoxins act only the sympathetic (Egdahl 1959 a and b Nykiel and Glaviano 1961 Spink *et* Levy and Blattberg 1967 Cavanagh *et al* 1970 Hall and Hodge 1971 Archer Bl Hinshaw 1975) but also the vagal system (Chien *et al* 1966, Levy and Blattberg 19 validity of this latter concept has been substantiated by our recent studies on the e endotoxin on cardiovascular reflex functions and circulation in dogs (Halinen, Ha and Sarajas 1975 Halinen 1976). The purpose of the present report is to describ sponding phenomena in cats. Such a comparative approach was considered useful s cardiovascular responses of cats to endotoxin seem to differ from those in dogs. For e



] Splenic sympathetic efferent discharge and aortic pressure curve before (A), 5 min (B) and 15 min after bolus injection of endotoxin E. ... to ... cat. The horizontal lines on each tracing indicate aortic ... levels of 100 and 0 mmHg

The splenic sympathetic efferentation exhibited a stepwise activation, the maximum, about -fold rise, became apparent at the end of the experimental period (Fig. 1). The relevant cordings obtained from an individual experiment are exemplified by Fig. 3.

The central venous pressure increased from the control level of 2.9 cm H_2O to 5.6 cm H_2O 0.05). This maximum level was attained to 5 min following the endotoxin injection. Acid–base balance and blood gas state were determined before the endotoxin injection and at 11 and 21 min after it. At 11 min the mean arterial pH had decreased from 7.40 ± 0.04 (S.D.) to 7.33 ± 0.03 (p 0.01), arterial P_{CO_2} from 30.4 ± 11.1 mmHg to 23.7 ± 7.1 mmHg (S.) and the arterial P_{O_2} from 139 ± 25 mmHg to 102 ± 18 mmHg ($p < 0.01$) and the plasma bicarbonate from 21.3 ± 3.2 mmol/l to 16.2 ± 2 mmol/l (p 0.01). At 1 min a recovery towards the control values was observed in all these parameters.

Discussion

Endotoxin evoked a drop of the arterial pressure within 60 seconds after the injection. This initial blood pressure fall is traceable to an endotoxin-induced pulmonary vasoconstriction being particularly pronounced in cats (Kuida *et al.* 1958. Kuida *et al.* 1961. Parratt 1973). The maximum pulmonary vasoconstriction ensues within few minutes after endotoxin injection (Parratt and Sturgess 1975). Its relief may be responsible for the transient rise of the arterial pressure observed at 3–4 min following the endotoxin injection in the present study (Fig. 1).

The aortic arch baroreceptor discharges decreased, and in some cats completely ceased, with falling arterial pressure. After the endotoxin injection, the aortic pressure was below the threshold for the aortic baroreceptors (Sagawa, Kumada and Schramm 1974), but the ... discharge rate did not decrease lower than to about 30 per cent of the control level. ... er only and ... re responses occurred in some cats. The changes of the

Fig. 1. Mean values (±S.E.) fo the heart rate, aortic pressures (systolic and diastolic), the splenic(and cardiac (●) sympathetic efferent discharges, and the aortic arch baroreceptor (○) discharges in 9 c[ats] given a bolus injection of *E. coli* endotoxin (1 mg/kg i.v.) at the zero time. Horizontal lines indic[ate] control level of each parameter. Statistically significant changes are labelled with asterisks. (P 0.05 P<0.01 P<0.001)

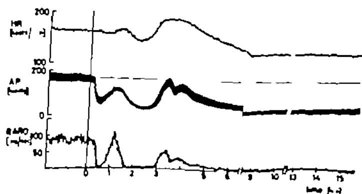
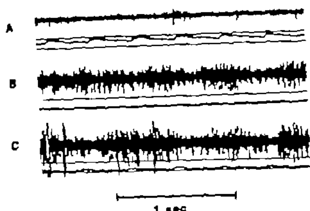


Fig. 2. Changes in the heart rate, aortic pressure and aortic arch baroreceptor discharge rates induced by a bolus injection of endotoxin *E. coli* L to a cat at zero time. Horizontal lines indicate the control level of each parameter.



1 Splenic sympathetic efferent discharge and aortic pressure curve before (A), 5 min (B) and 15 min after bolus injection of endotoxin *E. coli* to cat. The horizontal lines on each tracing indicate aortic pressure levels of 100 and 0 mmHg

The splenic sympathetic efferentation exhibited a stepwise activation the maximum, about fold rise, became apparent at the end of the experimental period (Fig. 1). The relevant cordings obtained from an individual experiment are exemplified by Fig. 3

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Heart rate [beats/min]
200
160
120
Aortic pressure [mmHg]
80
40
0
HR
AP
Autonomic discharges [fraction of control al]
5.0
4.0
3.0
2.0
1.0
0
8.49 ± 5.89
splenic
cardiac
baro
0 2 4 6 8 10 12 14
time

Fig. 1 Mean v lues (± S.E.) for the heart rate, aortic pressures (systolic and diastolic), the sp and cardiac (●) sympathetic efferent discharges, and the aortic arch baroreceptor (O) discharges given a bolus injection f *E. coli* endotoxin (1 mg/kg i.v) t the zero time. Horizontal lines control level of each parameter Statistically significant cha ges are labelled with asterisks (P < 0.01 P < 0.001)

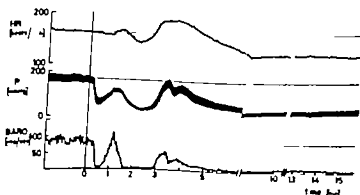
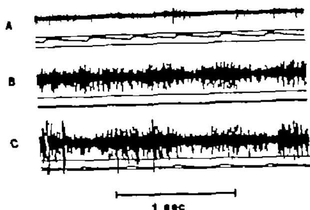


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The splenic sympathetic efferentation exhibited a stepwise activation, the maximum, about -fold rise, became apparent at the end of the experimental period (Fig. 1). The relevant recordings obtained from an individual experiment are exemplified by Fig. 3

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Fig. 1. Mean values (± S.E.) for the heart rate, aortic pressures (systolic and diastolic), the splenic (■) and cardiac (●) sympathetic efferent discharges, and the aortic arch baroreceptor (O) discharges in 9 given a bolus injection of *E. coli* endotoxin (1 mg/kg i.v.) at the zero time. Horizontal lines indicate control level of each parameter. Statistically significant changes are labelled with asterisks (* P ... ** P < 0.01 *** P < 0.001)

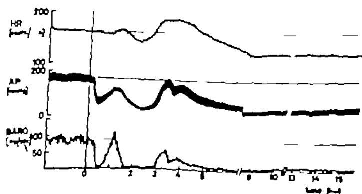


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sympathetic discharge rates inversely followed those of the arterial pressure. The cardiac sympathetic activation, however, seemed to be delayed when compared to the arterial pressure fall. This agrees with the observation of Parrat (1973) who reported an initial decrease of cardiac contractility and its recovery within 1–3 min of the initial endotoxin-induced hypotension.

The heart rate began to increase after a slight initial reduction and reached its maximum at 3 min after the injection. An initial bradycardic reaction has been observed in cats in response to endotoxin injection (Parrat 1973), and this bradycardic episode is followed by tachycardia (Parrat and Sturgess 1975). The initial bradycardia may arise from the effect of pentobarbital, for in pentobarbitalized dogs bradycardic responses to endotoxin injection have been frequently reported (Levy and Blattberg 1967). A vagal activation in canine endotoxemia was observed in a previous study (Halinen, Hakumäki and Sarajas 1976, Halinen 1976). If the phenomenon exists in cats, it might explain the decreasing heart rate after 3 min despite of the sympathetic activation in the present work.

The splenic efferentation gradually increased up to about 10-fold. Like the cardiac efferent discharge, the splenic efferentation mirrored the arterial pressure changes. The splenic and cardiac sympathetic fibres are known to respond in a parallel way to the baroreceptor input. The gain of the baroreceptor–sympathetic system to the spleen was maximally 2-fold greater than the gain to the heart at aortic pressure levels below 100 mmHg. This is more than that in cats under pentobarbital (1.6-fold) reported by Ninomiya, Nisimaru and Irisawa (1971) within an aortic pressure range of 100 to 150 mmHg.

Slight but significant changes towards metabolic acidosis and a fall of arterial P_{O_2} occurred in the cats of the present study. These phenomena have been reported also earlier in dogs and cats (Hardaway *et al.* 1961, Dedichen 1972, Parrat and Sturgess 1975, Halinen 1976). They are ascribable to decreased cardiac output (Gilbert 1960) and to changes in the pulmonary circulation leading to pulmonary edema in response to endotoxin (Kuida *et al.* 1961).

The average cardiovascular responses to endotoxin in cats were delayed when compared to those in dogs under morphine-chloralose anesthesia (Halinen 1976). Also the cardiac sympathetic efferentation was activated substantially later in cats as well. Despite of the distinct tachycardia elicited in dogs and the transient tachycardia in cats, the responses were nevertheless uniform in general. The splanchnic sympathetic activation presumably occurs also in dogs as shown by intestinal blood flow measurements by Lillehei *et al.* (1964) and Dietzman *et al.* (1973).

The hemodynamic responses to endotoxin are likely to result from the release of vasoactive amines (*cf.* Greenway and Stark 1971, Lefer 1973, Elin and Wolff 1976) into the circulation in response to endotoxins. Possibly the hemodynamic changes secondarily activate the sympathetic nervous system through cardiovascular receptors. The sympathetic system is nevertheless in competition with potent vasodilator substances, and the increased sympathetic drive may actually lead to a further impairment of the peripheral circulation.

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Acta physiol. scand. 1977 101 270–277

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Influence of Muscle Length on the Force–Velocity Relation of K^+-contractures in Smooth Muscle from Rabbit Urinary Bladder

By

BENGT UVELIUS

Received 24 March 1977

Abstract

UVELIUS, B. *Influence of muscle length on the force-velocity relation of K-contractures smooth muscle from rabbit urinary bladder* Acta physiol. scand. 1977 *101* 270–277

Force-velocity relations of K-contractures of longitudinal smooth muscle from rabbit urinary bladder were studied by isotonic quick release at 37°C. In order to minimize the influence of parallel elastic the study was limited to the rising part of the length-tension curve. The force-velocity data fitted well Hill's equation. The in situ length of the strip at a bladder volume of 10 ml is called L_{10}. This length 50% of that at which maximum active tension is developed. At L_{10} V_{max} was 0.29 muscle lengths per sec and it was estimated to be 0.36 lengths/s at optimum length. Constant b in Hill's equation had a value 0.052 L_{10}/s and it was unaffected by length changes over the interval 0.69 L_{10} – 1 44 L_{10}. At L_{10} P 0.17 I the interval given above, a/P decreased with increasing length in proportion to the increase P_0, indicating that a was also length independent. According to Hill's equation $[V = b(P - P)/(P + a)]$ should increase in proportion to $(P - P)$ when the muscle length is increased if and b are constants. a linear relation was found at shorter lengths but at lengths close to or at the length for maximum tension, V increased more than $(P - P)$. Two possible explanations were considered, firstly that b increased, and secondly that the load on the contractile element could be less than P due to an influence of the considerable tension in the parallel elastic element at these lengths. The series elastic recoil of the active muscle amounted to 3–4% of the muscle length when released to zero tension.

The relationship between force and shortening velocity in muscle is an important characteristic of the contractile machinery. It can be described mathematically by Hill's equation (Hill 1938) $v(P + a) = b(P - P)$ which represents a rectangular hyperbola with asymptotes at $v = -b$ and $P = -a$, and a P axis intercept at P_0, the isometric force. Hill's early work on frog skeletal muscle indicated that the constants a and b which can thus be obtained from the force-velocity relation by purely mechanical expts., reflected directly the rate of extra energy liberated during shortening (mechanical work + shortening heat). Later investigations have cast doubt on this direct relation between thermal and mechanical constants (see *e.g.* Simmons and Jewell 1974). However the force–velocity relation is still of great importance in view of its mere mechanical implications. It illustrates directly the power output from the muscle as a function of load and determines *e.g.* the rate at which the active muscle may shorten in response to a change in load

etching of skeletal muscle to sarcomere lengths above 2.0 μm causes the isometric [t]o decline, whereas the maximal shortening velocity (V_{max}) remains almost un[chang]ed (Gordon, Huxley and Julian 1966). On the rising phase of the length-tension curve, changes roughly in proportion to P_o. The constants a and b in Hill's equation are in [...]rpon unaffected by length changes (Abbot and Wilkie 1953). For heart muscle P [and] V_{max} have been found to increase proportionately with length on the ascending part [of th]e length-tension curve while the constants a and b remain unchanged (Nilsson 1972).

[Th]e mechanics of smooth muscle has been studied a great deal over the last few years. [Qualit]atively smooth muscle behaves as other muscle types in that the force-velocity relation [can b]e fitted to the Hill equation, but V_{max} is much lower than in striated muscle (see *e.g.* [...]ens, Kroeger and Mehta 1969 Gordon and Siegman 1971 a, Herlihy and Murphy [...] Peterson 1974, Hellstrand and Johansson 1975). There is, however not enough informa[tion] concerning the question of how the force–velocity curve in smooth muscle is affected [by le]ngth changes although some studies ha e been done (Gordon and Siegman 1971 a, [...]r *et al.* 1975) It seems that further systematic investigation is necessary

[Th]e aim of the present expts. on smooth muscle from rabbit urinary bladder was to study [the ef]fect of length on V_{max}, P and the constants a and b in Hill's equation. This preparation [...]tains low resting tension for quite large changes in length (Uvelius 1976 b). The expts. [were] performed using the isotonic quick release method on K -activated strips. Some of the [result]s have been reported in preliminary form (Uvelius 1976 a).

Methods

[...]ts of either sex weighing 2.5–3 kg were killed by cervical fracture and the urinary bladder was [dissect]ed out, emptied and transferred to Ca²⁺-free Krebs solution (composition in mM NaCl 122, [KCl 4.]73, $NaHCO_3$ 15.5, KH_2PO_4 1.19, $MgCl_2$ 1.19 and glucose 11.5). After 1 h in this medium at 4°C, [...] seemed to cause complete relaxation, the bladder was filled to 10 ml with the Ca-free solution and [a seg]ment of the longitudinal smooth muscle coat was marked out, dissected free and transferred to the [...] bath in the apparatus described below As in the earlier studies (Uvelius 1976 a, b) the length of the [segm]ent at bladder volume of 10 ml will be referred to as L_{10}.

[Th]e recording apparatus was essentially the same as described earlier (Johansson 1973) except that the [musc]le now was fixed with small metal clips at both its ends. One clip was connected to an isometric force [trans]ducer and the other to light lever (equivalent mass 150 mg with the muscle clip attached). The lever [...]ected photoelectric displacement transducer and could be clamped or released by an electromagnet[...]ated stop By means of spiral spring the load on the lever could be varied over the range of 0 to 10^{-4} N [In so]me of the expts. the initial oscillations of the lever after release were reduced by letting move in [a ...]er that containing drop of oil of suitable viscosity In other expts the lever was undamped There [were] no differences in the force–velocity characteristics of the muscles as obtained from recordings with [and] without damping The output from the apparatus was recorded on linear direct-writing oscillograph [...]ens MX4)

[Th]e muscle was allowed to accommodate for 1 h in physiological salt solution of the following composi[tion i]n mM NaCl 120, KCl 6.0, $MgCl_2$ 1.2, $CaCl_2$ 2.5, glucose 11.5 and tris(hydroxymethyl)aminomethane [...]ma Base, Sigma Chemical Co) 35 The solution had been titrated with HCl at 37 C to pH of 7.4 [and wi]ll in the following be referred to as "Na-tris, 2.5 Ca²⁺ After the accommodation period the muscle [was] transferred to solution with the same composition except that $CaCl_2$ was omitted (Ca-free Na-tris). [Spon]taneous mechanical activity then rapidly disappeared. Preload was kept at bout 3–4 of maximum [...] tension unless otherwise stated After 5 min the muscle was transferred to K-tris, 2.5 Ca²⁺" / [solu]tion with the same composition as Na-tris, 2.5 Ca²⁺ except that all NaCl was replaced with equimolar [amou]nts of KCl In this solution the muscle develops steady contracture The preparation was kept in

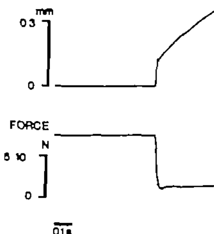


Fig. 1 A typical recording of an undamped q... lease. The muscle is released from 7.5 to 1.4 mN (... panel). After the release the muscle recoils rapi... after some inertial oscillations it begins to shorten... slower rate (upper panel)

K tris, 2.5 Ca for 5 min and was then transferred to Ca-free, Na-tris for 5 min which produced ... plete relaxation. This time schedule was then maintained throughout the experiment. Quick r... against various loads were made during the steady contractures. All solutions were bubbled ... and kept at 37°C.

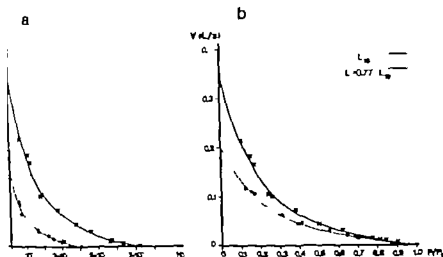
Results are given below as mean values ± S.E.

Results and Comments

Fig. 1 shows an original recording obtained for a quick release against a moderate ... during a steady contracture in K tris, 2.5 Ca + Immediately after the release the ... recoils rapidly and this event is followed by slower shortening.

The low preloads used in this study made it possible to leave the parallel elasticity ... consideration. According to Johansson, Hellstrand and Uvelius (1977), the length c... after the release can be described as consisting of 3 components an immediate ... of the series elastic component (SE) followed by a velocity transient lasting 50–75 m... finally a slower phase in which the contractile element is fully adjusted to the lo... order to avoid the influence of the velocity transient shortening velocity was measure... ms after the release. To measure shortening velocity at a fixed time like this may ... that the measurements are taken at different lengths of the contractile element. These ... tions did however not exceed 3 % of the muscle length in this study. Between P and ... the difference in length did not exceed 1 %. The recoil of the SE element was in all m... 3–4 of the muscle length when the fully activated muscle preparation was releas... zero load

The relation between load and velocity at all lengths studied was found to fit well wi... Hill (1938) equation. By plotting the experimental data as $(1 - P/P_o)/V$ vs. P/P and app... linear regression analysis, V_{max} and the constants a and b could be calculated. The soli... in Fig. 2 a shows the configuration of the hyperbola that fitted best to the data for one m... at L_{1o} when examined by such linearization of Hill's equation. After the series of rel... at L_1 the length of the muscle was passively changed and the whole sequence was repe... The lower curve of Fig. 2 a shows the force–velocity relation at L = 0.77 L_{1o}. It is seen ... V_{max} expressed in mm/s is lower at the shorter muscle length. A... velocity is



FV-relations for one muscle studied at L_{10} and 0.77 L_{10}. The hyperbolas that fitted best with the ... examined by ... linearization of Hill's equation are shown in the figure. 2 ... shows shortening ... expressed as mm/s and force in mN. In 2 b shortening rate is expressed as the actual muscle length ... force ... expressed relative to the isometric tension at the respective length.

fraction of the actual muscle length per second, a lower value of V_{max} is obtained at the ... length (Fig. 2 b). In this figure P is normalized as P/P_0. In most expts. it was possible ... obtain 2 complete force–velocity curves from the muscle. For the 8 muscles studied in this ... V_{max} at L_{10} was 0.29 ± 0.03 L_{10}/s.

... earlier study has shown the effects of length on isometric contracture force in the ... smooth muscle of the bladder (Uvelius 1976 b). To see if ... change of muscle length would ... ence V_{max} (expressed in mm/s) and P ... to the same extent, the quotient between the ... values at the greater and the shorter length examined in the individual experiment of the ... present study was divided by the corresponding quotient for V_{max}. If the relative change in ... was paralleled by the same relative change in V_{max} the result should be 1. A figure of ... 0.04 (n ... 8) was obtained suggesting that V_{max} is, if anything, more sensitive than ... variations in length over the range examined here (see further below).

The value of the constant *b* was found to be 0.052 ± 0.008 muscle lengths per second at L_{10} (... 8). The effect of length changes on constant *b* is shown in Fig. 3 A. The value of ... L_{10} for the individual muscle was set to 100% and the values obtained at other lengths ... expressed as percent of the L_{10} value. It is seen in the figure that there was no systematic ... range of *b* in response to variations in length. The mean length of the stretched muscles ... 1.33 L_{10} ... 0.05 L_{10} (... 5) and for these the constant *b* became 0.054 ± 0.007 L_{10}/s. ... thus appears that *b* is essentially independent of the degree of stretch for the length interval ... studied in this preparation.

The constant ... and P ... are both dependent on the cross-sectional area of the muscle and ... is therefore appropriate to examine the quotient between the two values. In the present ... study a/P ... was found to be 0.17 ± 0.02 (n ... 8) at $L = L_{10}$. When the muscles were stretched,

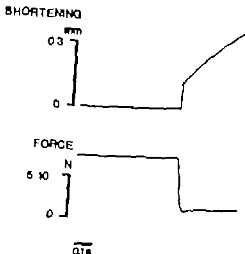


Fig. 1. A typical recording of an unloaded quick release. The muscle is released from 7.5 to 1.4 ... panel). After the release the muscle recoils rapidly; after some inertial oscillations it begins to shorten at a slower rate (upper panel)

K tris, 2.5 Ca^{++} for 5 min and was then transferred to Ca-free, Na tris for 5 min which produced complete relaxation. This time schedule was then maintained throughout the experiment. Quick releases against various loads were made during the steady contractures. All solutions were bubbled with ... and kept at 37 C.

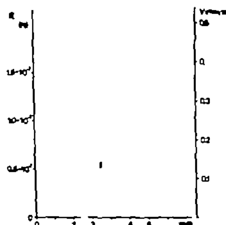
Results are given below as mean values ± S.E.

Results and Comments

Fig. 1 shows an *original recording* obtained for a quick release against a moderate load *during a steady* contracture in K tris, 2.5 Ca^{++}. Immediately after the release the muscle recoils rapidly and this event is followed by slower shortening.

The low preloads used in *this study* made it possible to leave the parallel elasticity out of consideration. According *to* Johansson, Hellstrand and Uvelius (1977) the length change after the release can be described as consisting of 3 components: an immediate recoil of the series elastic component (SE) followed by a velocity transient lasting 50–75 ms and finally a slower phase in which the contractile element is fully adjusted to the load. In order to avoid the influence *of the* velocity transient, shortening velocity was measured ... ms after the release. To measure shortening velocity at a fixed time like this may ... that the measurements are taken at different lengths of the contractile element. These variations did however not exceed 3 % *of the* muscle length in this study. Between P and P the difference in length did not exceed 1 %. The recoil of the SE element was in all muscles 3–4 of the muscle length when the fully activated muscle preparation was released to zero load.

The relation between load and velocity *at all* lengths studied was found to fit well with Hill (1938) equation. By plotting the experimental data as $(1 - P/P_0)/V$ *vs* P/P and applying linear regression analysis, V_{max} and the constants *a* and *b* could be calculated. The solid line in Fig. 2 a shows the configuration of the hyperbola that fitted best *to the* data *for* one muscle at L_{10} when examined by such linearization of *Hill's equation*. *After the* series of releases at L_1 the length of the muscle was passively changed and the whole sequence was repeated. The lower curve of Fig. 2 a shows the force velocity relation at $L \sim 0.77$ L_{10}. It is seen that V_{max} expressed in mm/s is lower at the shorter muscle length. Also if velocity is expressed



4 Results from one experiment showing P – P circles) and V (crosses) as functions of muscle V the shortening velocity against load P was held constant (in this expt. 0.3 · 10^{-3} N). d V increase in proportion up to muscle 5.5 mm. Above this length V increases P P L_{10} as for this muscle 4.4 mm. r details see text.

of 0.29 lengths/s at L_{10}. The maximal active tension at optimal length is 13 N/cm² 1976 a, b). These differences between smooth muscles from various sources may e due to differences in gross tissue structure (presence of other tissue components ooth muscle, inhomogeneity of cell orientation, variations in size of extracellular c.) but they may also reflect biochemical and ultrastructural variations in the con- ystems (see Rüegg 1971).

eletal muscle both V_{max} and P increase with muscle lengths up to where P is maxi- rther stretching decreases P whereas V_{max} is unaltered (Gordon, Huxley and Julian Due to the considerable passive tension of the strips of urinary bladder at lengths than 1.8 L_{10} it was only possible to study the force-velocity relations on the rising the length-tension diagram. For length changes between 69 and 144 per cent of L_{10} nd P changed to about the same relative extent. This was seen both in recordings of he force-velocity curves at different lengths and by the fact that the shortening velocity a given load, P was proportional to (P – P). At larger lengths, V was found to in- faster than P P (see Fig. 4). The reason for this greater increase is not clear. It be related to changes in one or both of the parameters *a* and *b* but it is also possible e force carried by the contractile element is simply less than the afterloaded P at these s due to an influence of the parallel elasticity. It is thus possible that the true relation m V_{max} and P is linear also at these lengths. At L V_{max} was 0.29 muscle lengths/s. al active tension is attained at 2.0 L_{10}. At this length, P is 2.5 times P at L_{10} (Uvelius b). V_{max} at the optimal length may then be estimated to be about 0.36 lengths/s. above expts. suggested that *a* and *b* are constants independent of length in the range m 69 and 144 per cent of L_{10}. For *b* this is also supported by the results shown in A. The constant *b* was 0.052 ± 0.008 L_{10}/s at L_{10} and 0.054 ± 0.007 L_{10}/s at 1.33 L_{10}. r reported values for *b* in smooth muscle may be exemplified by the following data gths/s. 0.035 for cat intestinal muscle at 30°C (Meiss 1971), 0.02 for hog carotid artery C (Herlihy and Murphy 1974) and 0.05 for guinea pig taenia coli at 36°C (Mashima Handa 1969).

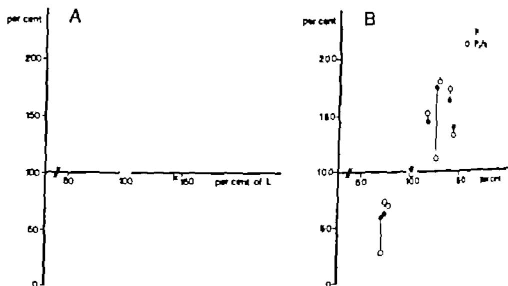


Fig. 3 A Effect of length changes on constant b in Hill's equation. B Influence of length chang… isometric force (P_o) and P_o/a. The values of the variables were set to 100% at L_{18} and the values ob… at other lengths were expressed as percent of the L_{18} values. P and P_o/a seem to be affected … length changes in the interval studied (0.69 L_{18} – 1.44 L_{18}), whereas no systematic effect on b is …

a/P was found to decrease. In order to see if this decrease at longer lengths was … due to the increase of P_o, a plot of P_o/a and P was made. The value of both at L … has been set to 100 % in Fig. 3 B. If a itself is independent of length then P_o/a and P shou… affected to the same degree by changes of the muscle lengths and according to the fi… this seems to be the case.

The Hill equation can be written as V = [b/(P + a)] (P − P). Then, if a and b are … independent of length as the above expts. indicate, the velocity of shortening against a … load, P will be proportional to (P − P) when P is varied by changes in the muscle len… In a separate set of expts the preparations were released against the same load during … tractures elicited at several different lengths. Fig. 4 summarizes results from one of … expts. It is seen that with increasing length V and (P − P) increase proportionately … about 1.25 L. Above this length V increases faster than (P − P). This means that … about 1.25 L V is a linear function of (P − P) and that above this value b/(P + a) incre… This finding will be commented upon in the discussion.

Discussion

Maximal shortening velocity varies greatly between different smooth muscles. Strips … hog carotid artery show a V_{max} of only 0.12 muscle lengths per second at 37°C (He… and Murphy 1974) whereas values of 0.74 lengths/s have been obtained in the rat p… vein (Hellstrand and Johansson 1975). On the other hand the preparation of hog ca… artery develops high isometric tension (P 18 N/cm²) whereas the rat portal vein … reaches 3–4 N/cm vessel wall or 8–10 N/cm muscle layer (Johansson 1976). Strips of lo… tudinal smooth muscle from rabbit urinary bladder have according to the present st…

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The absolute values of the constant *a* of a preparation is dependent on the tension de ment which is in itself dependent on cross sectional area. It is common therefore to co a/P instead of *a* At the optimal length, a/P is 0.24 for cat intestinal muscle at 30°C 1974) 0 18 for hog carotid artery (Herlihy and Murphy 1974) and 0.21 for dog tra (Stephens *et al* 1969) both at 37 C. In our preparation P_o/a and P varied to the same with the preparation length (Fig. 3 B) and thus *a* was also a constant within the interval studied. This is in accordance with the findings of Abbot and Wilkie (19 skeletal muscle Nilsson (1972) on papillary muscle and Gordon and Siegman (on rabbit taenia coli. The quotient a/P is, in contrast to *a* not constant it decr greater lengths as P increases. Thus no characteristic a/P can be given but at the value is 0 17 ± 0 02. The optimal length for active tension development is, as de earlier (Uvelius 1976 a, b), about 2.0 L , at which the muscle develops about 2 more active tension than at L . Hence a/P at this length should be about 0.07 if a results indicate is a length independent constant. Gordon and Siegman (1971 a) fo significant change in a/P with length in their taenia coli preparation but their va in length were smaller than in this study

In summary it can be said that the value of *b* found in this study is in good a with those obtained for other smooth muscles, whereas the estimated a/P at optimal is considerably smaller It could be possible that *a* at the optimal length for this prep might be greater than at the lengths studied or that the high resting tension prev some of the studies by others could influence *a* Finally the mode of activation could h the results. Hellstrand and Johansson (1975) and Murphy (1976) have noted that ac by K high solution as in this study increases the curvature of the force-velocity in comparison to that found in tetanic activation and this would imply a lower contractures.

The maximum extension of the series elastic component was 3–4 % of the muscle a value which is lower than the ones reported earlier for vertebrate smooth muscle for other preparations vary between 20 % for rabbit taenia coli (Gordon and Siegman and 4.3 % for rabbit mesotubarium (Meiss 1975) These considerable variations part be due to different amounts of injured tissues in the preparations. It is also that differences in apparatus compliance might contribute. That there exist true dif between SE behaviour for different smooth muscles is, however shown by Johanss (1977) In their study a comparison was made between the SE stiffness for rabbit bladder smooth muscle and rat portal vein The SE element of the bladder prep were found to be about 3 times stiffer than that of the portal vein at any force le preparation used in the present study is histologically very homogeneous. The lo of the cells have the same direction as the whole muscle and this might be one reason stiff SE but further study on this point is necessary

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Thanks are due to Ina Gunnarsson, Monica Heidenbolm and Monica Lundahl for techn secretarial assistance.

›n and Albrecht 1975). Another indirect method, Xe-clearance, requires a homogeneous ution to be valid (Kety 1951). So far there is no conclusive evidence for a homogeneous sion throughout the testis. The use of radioactive microspheres would offer an advan- o the technique mentioned above, since the method can be applied to a surgically intact i and it requires no homogeneously perfused vascular bed. In addition, this method has shown to be suitable to measure flow in hyperperfused tissues (Janson and Albrecht . The technique was modified for use in small laboratory animals by Rudolph and Hey i (1967) and was recently applied to the rat (Bruce 1976). In the literature there is no no full report on its use for testicular blood flow measurements.

e purpose of the present study was to set up a technique for measuring testicular blood in the rat by use of radioactive microspheres and to compare this method with the nonly used Xenon-133 clearance technique and with direct measurement of spermatic us outflow. While there is no information available on the distribution of blood flow rious segments of the testis, an additional purpose of the study was to perform such urements.

Materials and Methods

ut 80 male rats of the Sprague-Dawley strain (Møllegaard-Hansen, Ejby Denmark), eighing be- i 300 and 400 g, were kept in controlled environment. Food and ater were available *ad libitum*. esthesia All animals ere anesthetized by sodium pentobarbitone (Nembutal Abbot, U.K.) in of 40 mg/kg p. given as single injection. During each expt. the rat as kept supine on heating

…nique for testicular blood flow measurement

…t measurement 15 animals were laparotomized and the intestine as gently pulled upward and to ft and covered ith cloth soaked in arm saline. By this procedure the right spermatic artery and were exposed. By use of polyethylene catheter (PE 50, Intramedic, Clay Adams, U.S.A.) the spermatic was cannulated at least 5 mm from its entry into the caval ein. No ligation of the spermatic ein ...ted to the cannulation as necessary since the PE 50 catheter fits very tightly into the ein and no age of blood as possible. Care as taken to avoid manipulation of the spermatic artery. Blood flow ...urement was started 2 3 min after the cannulation. The catheter 3–4 cm in length, as kept in d position ith its open end at the level of the caval vein. The spermatic venous blood as collected ...weighed glass tubes for one-minute period after hich the testicular artery was ligated through ...ously made midscrotal incision (see Fig. 1). Immediately following the ligation, the spermatic venous flow as measured for another one-minute period. The blood olume was calculated by use of the of weight of rat hole blood, 1.05. By subtracting the blood volume collected after ligation of the ...cular artery from the total spermatic venous outflow testicular blood flow as calculated. During whole experimental period the blood losses ere substituted for by giving an equal volume of Macrodex ...rmacia AB, Sweden) into the external jugular ein.

Xenon-133 clearance. Xenon-133 dissolved in sterile saline as obtained from AB Atomenergi, Studsvik, eden. Approximately 20–40 μCi in 30–40 μl saline as injected percutaneously into the right testis of rat ith gastight Hamilton syringe. A Na-I crystal detector (Frieseke-Hoepfner 421 A) as used th 10 mm ... collimator (Frieseke-Hoepfner 49 A scaler) and recording as made on Hewlett- ckard 7127 A strip chart recorder. After background subtractions the recordings were plotted on semi- arithmic paper and the calculated $t_{1/2}$ for the washout curve as introduced into the following formula, given by Kety (1951) in order to obtain blood flow

$$F = \frac{\ln \quad K \quad 100}{1/2}$$

he partition coefficient, K, used as 0.85 (Betchell *et al.* 1966).

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Methodological Aspects of Testicular Blood Flow Measurements in Rats

By

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Abstract

DAMBER, J. E. and P. O. JANSON. *Methodological aspects of testicular blood flow measurements in rats.* Acta physiol. scand. 1977. *101.* 278–285.

3 techniques for the measurement of testicular blood flow in anesthetized adult rats were compared. Direct measurement of testicular venous outflow yielded values more than 3 times lower than those obtained with Xe-133 clearance and radioactive microsphere techniques due to the surgical procedures involved in spermatic venous cannulation. There was an agreement between flow values obtained with Xe-133 clearance (17.8±3.5 ml/100 g min) and radioactive microspheres (19.9±5.5 ml/100 g min). A homogeneous distribution of microspheres to different segments of the testis indicates that Xe-133 clearance is a suitable technique for testicular blood flow measurements. However for some experimental purposes the radioactive microsphere technique is more versatile than Xe-133 clearance because of its capacity of measuring several organ flows simultaneously.

Blood is the major route for the transport of androgens from the testes to the rest of the body (Setchell 1970). Since perfusion rate is of paramount importance for testosterone secretion (Eik-Nes 1964) many factors influencing the testicular blood flow may affect testicular hormone secretion. In studies of testicular endocrine function it is therefore important to measure circulatory variations in an accurate way.

Many techniques have been used to measure testicular blood flow in different species such as direct measurement of venous outflow, Xenon-133 clearance, indicator fractionation (Setchell 1970), and, recently by use of a miniature friction flowmeter (Jaffe and Free 1...). The results reported are approximately of the same magnitude but information is lacking regarding the prerequisites for the use of a particular method in relation to other techniques. For instance, direct techniques involving venous cannulation and the use of flowmeters may disturb testicular blood flow in a way similar to that reported for the ovary (Janson and Selstam 1975). Also, indirect techniques, like, for instance, indicator fractionation (Sapirstein 1958) may yield erroneous results due to an incomplete extraction of the indicator from the blood to the tissues, as was recently shown for abunda[illegible] ovarian [illegible]

I. Testicular blood flow estimated by different methods in anaesthetized adult rats.

od	No.	Testicular blood flow (ml/100 g min)
t venous cannulation	15	5.9±4.3
n-133 clearance	25	17.8±3.5
active microspheres	25	19.9±5.5*

s are given as mean ± S.D
n arterial pressure during measurements: 96±25 mmHg.

ght testis, epididymis and fat along the vascular pedicle were dissected and counted for radioactivity her ith the spermatic venous blood. The number of microspheres in the blood, divided by the number tissues, was taken as an estimate of the degree of arterio-venous shunting of spheres.

testes, from rats infused with microspheres, ere cut ith sharp scalpel, in 4 segments from the and to the distal pole. The segments thus obtained contained different proportions of centrally and ferally located testicular tissue. Each segment was then counted separately for 5 min and the number heres per mg testicular tissue was calculated.

Results

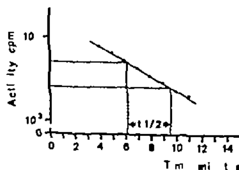
mparison between different techniques (Table I)

ect measurement. The testicular blood flow was 5.9±4.3 ml/100 g min (mean ± S.D.). this preparation, 46.2±15.8% (mean ± S.D.) of the total venous outflow in the right rmatic ein represented testicular blood flow the rest originating from the epididymis d fat and connective tissue along the vascular pedicle.

Xenon-133 clearance. The blood flow in the right testis estimated by this method was 8±3.5 ml/100 g min (mean ± S.D.). Fig. 2 shows a typical clearance curve for Xenon-3 injected percutaneously into the testis. A stable linear curve was obtained after 3–4 min d lasted at least for 7–8 min.

Radioactive microspheres. Testicular blood flow as determined by radioactive microspheres s 19.9±5.5 ml/100 g min (mean ± S.D.). Blood flow values for the epididymis, ventral ostate and kidneys were 1..6±4.22, 37.6±18.7 and 431.6±139.7 ml/100 g min respectively ean S.D.).

Since it is known (Buckberg *et al.* 1971) that the variability of distribution of spheres to



. 2. A typical clearance curve for Xenon-133 ected percutaneously into the right testis of an ult rat.

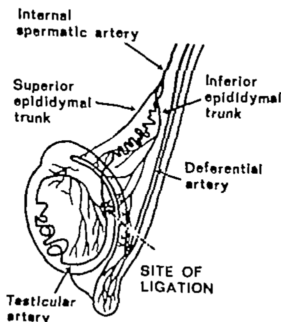


Fig. 1 Schematic illustration of the arterial to the testis and epididymis in the rat The ligation of the testicular artery during the measurements is indicated in the figure.

Radioactive microspheres. Radioactive "carbonized" microspheres labelled with ytterbium 169 and scandium 46 (^{46}Sc) and with a diameter of 15±5 μm were obtained from 3 M Co. (St. Paul, U.S.A.) suspended in 20% (w/v) dextran. The initial specific activity of ^{169}Yb was 10.07 mCi/g and ^{46}Sc was 8.63 mCi/g. A drop of detergent was added to inhibit aggregation of spheres. Around spheres were transferred t glass chamber holding a volume of 0.9 ml. The design of the chamber and handling of it were described in detail by Rudolph and Heymann (1967).

In 25 rats, catheters (PE 50) were inserted into the right brachial artery the tail artery and into the ventricle o the ascending orta via the right or left common carotid artery The catheters were filled a solution of heparin and saline (1 10). The right brachial arterial catheter was connected to a St P 23 AC transducer connected to a Grass Model 7 Polygraph. The glass chamber containing the suspension of spheres in dextran was vigorously agitated using a high-frequency mechanical stirrer and the suspension was flushed into the heart or ascending aorta for 30 s with 1 ml saline. 15 s before during, and for 15 s after termination of the infusion of microspheres, blood was withdrawn at constant rate (0.1 ml in the tail arterial catheter This reference sample was withdrawn into 2 ml disposable plastic syringe attached to pump adapted f aspiration on each rat the first using ^{169}Yb labelled spheres, under conditions. A second injection was made in 13 of the rats, using ^{46}Sc labelled spheres, after lowering blood pressure by giving an additional injection of barbiturate. After the second injection of microspheres the rat was killed with an overdose of sodium pentobarbitone and dissected Radioactivities of "reference sample" the testes and some other organs and tissues were measured in a automatic scintillation counter (Packard Auto-Gamma) Each sample was counted for 3 min. By counting standards of known number of microspheres from the actual batches, the number of spheres in different organs and tissues was calculated. Blood flow were then calculated as follows (Rudolph and Heymann 1967):

$$F = \frac{Q_{ref} \cdot N_{organ}}{N_{ref}}$$

where Q_{ref} = rate of withdrawal of the reference sample N_{organ} = number of spheres present in the organ and N_{ref} = number of spheres present i the reference sample

In order to obtain good accuracy with this technique blood flow values were not accepted unless the number of spheres in the organ to be studied exceeded 400 (Buckberg et al. 1971).

Another 15 rats were laparotomized and the right internal spermatic vein was cannulated, using a PE catheter t least 5 mm from the entry int the ca al vein. Blood drai ing the testis, the epididymis, fat and connective tissue along the vessels of the testis, was collected int small glasstubes for 5 min starting 2.5 min before an intracardiac infusion of Yb-labelled microspheres. After ified the

I Testicular blood flow estimated by different methods in anesthetized adult rats.

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venous cannulation	15	5.9±4.3
133 clearance	25	17.8±3.5
[illegible] microspheres	25	19.9±5.5[a]

[illegible] are given as mean ± S D
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Results

[illegible]parison between different techniques (Table I)

[illegible] measurement The testicular blood flow was 5.9±4.3 ml/100 g min (mean ± S D). [illegible] this preparation, 46.2±15.8 % (mean ± S.D) of the total venous outflow in the right [illegible]matic [illegible]ein represented testicular blood flow the rest originating from the epididymis [illegible] fat and connecti[illegible] tissue along the vascular pedicle.

Xenon-133 clearance The blood flow in the right testis estimated by this method was [illegible] 3.5 ml/100 g min (mean ± S D.). Fig. 2 shows a typical clearance curve for Xenon-[illegible] injected percutaneously into the testis. A stable linear curve was obtained after 3–4 min [illegible] lasted at least for 7–8 min.

Radioactive microspheres. Testicular blood flow as determined by radioactive microspheres [illegible] 19.9 5.5 ml/100 g min (mean ± S.D). Blood flow values for the epididymis, ventral [illegible]state and kidneys were 12.6±4.22, 37.6±18.7 and 431.6±139.7 ml/100 g min respectively [illegible] S D.).

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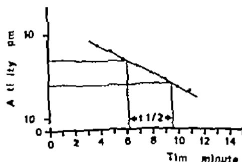


Fig. 2. A typical clearance curve for Xenon-133 injected percutaneously into the right testis of an adult rat.

TABLE II Distribution of 15 μm radioactive microspheres to different segments of 39 rat testes

Segment	Number of spheres per mg of wet tissue wt
A	1 8 ± 0.3
B	1.9 ± 0.3
C	1 7 ± 0.2
D	1 7 ± 0.2

Values given as means ± S.E. Analysis of variance gave no significant difference in sphere distribution different testicular segments.

any organ approximately follows a Poisson distribution accuracy of flow measurement the 95% level could be calculated as follows

$$\text{Accuracy } (\%) = \frac{1\ 96}{\sqrt{X}} \times 100$$ where X is the number of spheres in the organ to be measured

In the present study the mean accuracy was 3.9% for the testes, 8 6 for the epididymis 8 0 % for the ventral prostate and 0.9% for the kidneys. A paired comparison between flow per unit of weight in the right and the left kidney gave no statistically significant differences according to Wilcoxon s paired t test based on range (Siegel 1956). The same r were obtained for the right and left testis, and the right and left epididymis. Spearman correlation coefficient r (Siegel 1956) for testicular and epididymal blood flow was and was significant ($p < 0\ 01$). Blood pressure and heart rate were stable during the measurement of basal blood flows. Blood flow of the vascular pedicle, containing mostly fa 3 2 ± 0.3 ml/100 g min and blood flow of the lumbar muscle was 5 0 ± 0.8 ml/100 g These values are, however uncertain since the number of spheres in the tissues was 400 on several occasions.

Distribution of blood flow within the testis

The number of spheres per mg of wet tissue in the 4 segments of the testis is presented Table II No statistically significant difference in distribution of spheres to different te segments was obtained by analysis of variance.

Arterio-venous shunting

The passage of radioactive microspheres from the testis, epididymis and tissues alo vascular pedicle to spermatic venous blood was 0 9 ± 0 4 (Table III).

Relationship between arterial pressure and testicular blood flow during sodium pentobarbitone anesthesia

In 13 of the non-laparotomized rats, an additional i v dose of sodium pentobarbit given immediately after the first blood flow measurement using Yb-169 Five to 10 mi the first measurement the second one was performed with Sc-46. The change in bloo sure was −12.9 ± 8 8 % of the initial value, and the corresponding change in testicular flow was −12.6 ± 13 1 The correlation between arterial pressure and testicular fl low The testicular vascular resistance appeared to be increased by an additional sodium pentobarbitone (12.7 ± 10 6 of initial value).

[illegible] III. Passage of radioactive microspheres from testis, epididymis and tissues along vascular pedicle to spermatic venous blood.

Number of microspheres in testis, epididymis and pedicular fat (A)	Number of microspheres passed to spermatic venous blood (B)	A–V "shunting" (B/A( ))
2 393 ± 515.3	15.5 ± 6.0	0.9 ± 0.4

[illegible] are expressed as mean ± S.E.

Discussion

[illegible]cular blood flow measurements with radioactive microspheres in the rat hav[e] not been [repo]rted elsewhere. The flow values of the present study are of the same magnitude as those [repo]rted for the rabbit by Neutze *et al.* (1968) using the same method, and are also in agree[men]t with values reported by others using different methods (Setchell 1966, 1970, *Jaffe* and [...] 1972).

[W]hen using the microsphere technique it is essential that certain basal requirements are [ful]filled (Rudolph and Heymann 1972). The spheres should be evenly distributed in the [arte]rial blood stream, they should be completely entrapped in the tissues under study and, [fina]lly they should occur in sufficient numbers in the tissues to permit precision in the [mea]surements. In the present study the lack of significant differences in blood flow between [the] right and the left side in paired organs, indicates an even distribution of spheres and the [abs]ence of arterio-venous shunts larger than a diameter of 15 μm indicates a complete [en]trapment of spheres in the testis. Finally the number of spheres in the testis corresponded [to] a precision in the measurements of 2.5% [a]t 95% confidence level. Blood flow values [in] the present study of organs other than the testis were in agreement with those found by [oth]ers with both microspheres and other methods. Bruce (1976) used microspheres in young [fe]male rats and reported somewhat lower values for renal blood flow (217 ± 44 ml/100 g [...]) than obtained here for adult male rats. The values are, however of the same magnitude. [Set]chell *et al.* (1964) used the technique of Sapirstein (1958) and reported values of epi[didi]ymal blood flow very similar to those obtained here with microspheres. Anderson *et al.* [(19]67) described a method for measuring prostatic blood flow in dogs using Xenon-133 [cle]arance and reported [v]alues of 31–79 ml/100 g [...] min, which are in good agreement with [th]ose of the present study. Direct measurement of testicular blood flow by cannulation of [th]e spermatic [v]ein yielded values much lower than the other two methods used. However [...]sing radioactive microspheres in a preparation involving spermatic [v]ein cannulation [th]e results are in excellent agreement with the present direct measurements (Damber and [Jan]son, to be published). This indicates that the direct measurement reflects true testicular [bl]ood flow but that the actual flow is reduced by the procedures involved in the measure[m]ent itself.

Direct measurement of testicular blood flow involves laparotomy which reduces blood [fl]ow in the testis (Damber and Janson, to be published). Manipulation during the cannula[ti]on of the [v]ein is [al]so probably a factor reducing the testicular blood flow. As suggested

by Goding *et al* (1972), the flow can be reduced both by an increased venous pre to the cannulation and by arterial spasm caused by manipulation.

The percutaneous Xenon-133 technique for measuring testicular blood flow described by Wax (1971) Using dogs and rats he obtained clearance curves simil Clearance techniques are most suitable for homogeneous tissues where a uniform can be expected (Kety 1951) Although there is a rapid decay in radioactivity d first 1 to 2 min there is probably only one major component from the testis repre the stable linear curve obtained after 3–4 min. The lack of linearity during the fir of measurement is probably caused by the escape of Xenon-133 through the trac by the needle (Wax 1971). However Blombury and Waites (1968) interpreted t exponential clearance curves obtained by Krypton-85 clearance technique as a no perfusion of the testis However when using radioactive microspheres in the pre we were unable to find any differences in the distribution of spheres to different se the testis, indicating that blood flow throughout the organ is uniform and that X clearance should be applicable to measurements of testicular blood flow This w ported by the fact that, in the present study the Xenon clearance values were in g ment with those of the microsphere technique.

Sodium pentobarbitone is one of the most widely used anesthetics for anim ments. It is known to affect systemic and regional hemodynamics only slightly *et al* 1976) In our expts. barbiturate anesthesia resulted in stable blood pressure rate. Free and Jaffe (1972) found similar values for testicular blood flow in con as compared to barbiturate anesthetized ones. This indicates that testicular b values obtained during barbiturate anesthesia represent physiological flows, but the fall in blood pressure during anesthesia may be accompanied by a decrease in vascular resistance. In the present study an additional injection of barbiturate to an rats resulted in variable effects on blood pressure and testicular flow and inconsist on vascular resistance. Therefore, it is very difficult to make any conclusions about flow relationship for the testis with this design. However in most cases the testic flow was reduced when blood pressure decreased.

In summary the results of the present investigation indicate that 1) the operative associated with cannulation of the spermatic vein causes a significant decrease of blood flow which is of importance to consider when evaluating testicular andro tion rate in studies involving analyses of spermatic vein blood, 2) the percutaneo 133 clearance technique and the microsphere technique are both useful for studies flow regulation in the rat testes, 3) the distribution of flow to different segments uniform.

The particular aim of a study involving measurements of testicular blood flow considered when choosing between Xenon-133 clearance and microsphere techni simplicity economy and suitability of Xenon-133 clearance for "chronic" expts on certain occasions, be outweighed by the microsphere method because of its measure several organ blood flows simultaneously yielding an extensive pictu hemodynamic situation. The latter technique may for instance, be the one of ch trying to clarify whether a vascular effect of an agent is specific to the testis or no

...ial support ... obtained from The Medical Faculty of Umeå and from The Swedish Medical ...rch Council (B77 17X-04981-01). Professor Kurt Ahrén and Professor Hans Carstensen are thanked ...uable advice and access to experimental equipment. Skilful technical assistance by Miss Stina Öberg, ...Ann Kaufman and Miss Birgitta Svensäll is gratefully acknowledged.

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C-fibres had little, if any physiological role to play Thorén, Saum and Brown (1977) rat *in vivo* and Thorén and Jones (1977) in the rabbit *in vivo* have studied the char atics of aortic C-fibres in greater detail and found them to have higher thresholds and discharge frequencies than the medullated fibres. The C-fibres were thought to be mly anti-hypertensive in nature (Thorén and Jones 1977).

reflex effects of aortic baroreceptor C-fibres have also been studied. Thus electrical lation of these non-medullated afferents in the aortic and sinus nerves can evoke power pressor reflexes in cats (Douglas and Ritchie 1956 Douglas, Ritchie and Schaumann) and rabbits (Kardon, Peterson and Bishop 1975) with different efferent patterns from medullated fibres (Kardon *et al.* 1975).

iew of this w has studied some characteristics of C-fibres arising from the aortic in rabbits with chronic renovascular hypertension and compared them with medullated s arising from the same site.

Methods

l hypertension as induced in 6 rabbits by either polyethylene wrapping of the left kidney (1 expt) pping of the left renal artery (5 expts) using silver clips of 0 7 or 0 8 mm internal diameter according technique of Romero *et al* (1973) In all animals the contralateral kidney was removed 10 days later. lition another 14 rabbits were operated upon (5 polyethylene wrapping and 9 renal artery clipping) could not be included in the study because of either operative or postoperative deaths (7 rabbits) or ficient degrees of hypertension (mean arterial blood pressure (MABP) less than 125 mmHg).

ll rabbits blood pressure as checked pre-operatively hen the animals were fully conscious and dated, by means of direct needle puncture of the ear artery hich had been anaesthetized with few of subcutaneous local anesthetic Blood pressure as determined using the same method tw or times thereafter and finally one or tw days before the acute expt. was carried out.

n the day of the acute expt the rabbits were premedicated with diazepam (Valium 5 mg p) and thetized th sodium pentobarbital (30 mg/kg). A tracheal cannula was inserted, the animal was lysed with gallamine triethiodide (10 mg/kg) put on intermittent positive pressure ventilation. tional doses of anesthetic or gallamine were given as required.

he femoral artery was cannulated and the cannula passed up to the arch of the aorta for measurement ortic blood pressure A femoral vein was cannulated for administration of fluid and drugs The pH, O_2 and PO_2 were monitored and the respiratory pump adjusted accordingly Small doses of $NaHCO_3$ M/ml) ere given as required to correct any developing acidosis.

midline incision in the neck as made and the vagus and aortic nerves on the right side sectioned. left vagus was also sectioned and he left aortic nerve dissected free for distance of at least 3 cm llow adequate measurements of conduction elocity to be made. A pool as made from surrounding e and filled ith paraffin oil The left aortic nerve as placed on small plate and he nerve sheath oved under binocular dissection microscope Thin filaments ere teased free and placed on bipolar oridized silver electrode and connected to bipolar amplifier the output from hich was displayed on h an oscilloscope (Tektronix 502) and on an ultraviolet light (UV) recorder (ABEM 5651). In addition output from the amplifier as also led to loudspeaker and to ratemeter (time constant 0 4). The meter had discriminator so that could count either all spikes exceeding pre-determined level or l spikes ith amplitudes lying between tw pre-set levels. The output from the ratemeter was recorded Grass polygraph Model 7D It as possible to check the accuracy of the ratemeter by direct counting spikes in the UV recordings and this as done frequently throughout the expts

The conduction velocities of both the C-fibres and the medullated fibres were calculated from the time ay between the application of an electrical stimulus to the aortic nerv and the appearance of the evoked ential and from the distance between he stimulating and recording electrodes. In few cases the total ime between stimulus applied directly to the receptor in the aortic arch and the recording neck as measured

C-fibre and ts as obtained by recording from he filament during electrical the thorax. All filaments showing C-fibre activity were further

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Characteristics of Aortic Baroreceptors with Non-medullated Afferents Arising from the Aortic Arch of Rabbits with Chronic Renovascular Hypertension

By

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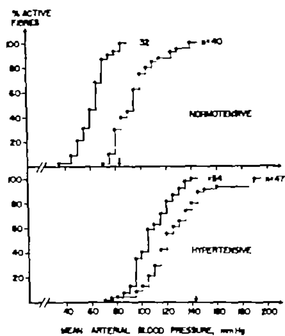
Abstract

JONES, J V and P N THORÉN *Characteristics of aortic baroreceptors with non-medullated afferents arising from the aortic arch of rabbits with chronic renovascular hypertension* Acta physiol. scand 1977 *101* 286-293

The characteristics of 47 non-medullated and 54 medullated fibres arising from the aortic arches of 6 hypertensive rabbits have been investigated The threshold for activation of the aortic C-fibres lay between [illegible] and 190 (mean 122) mmHg. The threshold for activation of 54 medullated aortic baroreceptors in the same animals was from 70 to 140 (mean 109) mmHg. At the awake mean arterial blood pressure (MABP) of these hypertensive rabbits (143 mmHg) all the medullated fibres were active as were 78 [illegible] of the C-fibres. In contrast in a group of normotensive animals 91 % of the medullated and only 78 % of the non-medullated fibres were active Pressure response curves were constructed for 19 C-fibres and 12 medullated fibres from the hypertensive animals At a MABP of 110 mmHg the firing in the non-medullated fibres was [illegible] Hz as compared with 19.0 H[illegible] the medullated fibres and [illegible]t the awake MABP mean firing [illegible] in the non-medullated fibres and 48.0 Hz in the medullated fibres. The firing at awake MABP [illegible] considerably higher in the hypertensives (9 0 Hz) as compared with the normotensives (1 1 H[illegible]). Thus these data indicate that although the aortic C-fibres are reset in chronic hypertension, they are reset less than the medullated aortic baroreceptors from the same animal. It is suggested that arterial baroreceptor C-fibres may have an important role in the tonic control of the circulation in hypertension.

There have been many studies over the years of the characteristics of the large myelinated fibres arising from the aortic arch and carotid sinuses in a variety of animals and even in man (Kirchheim 1976). Only recently have similar studies (Fidone and Sato 1969) begun to be performed on the small unmyelinated fibres arising from the baroreceptor regions These authors described the existence of baroreceptor C fibres although Landgren (1952) and Landgren, Neil and Zotterman (1952), many years earlier had described baroreceptor afferents in the sinus nerve with low signal to noise ratios which presumably were C-fibres More recently Coleridge *et al* (1973) recorded from aortic C-fibres in the vagus nerve of the dog and described them as having very high thresholds. Coleridge *et al* thought that

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[Fig.] 2. The bottom panel summarizes the [data] shown in Fig. 1. The percentage of [me]dullated and non-medullated fibres, [wh]ich have reached threshold, is plotted [agai]nst the MABP. The upper panel [sh]ows, for comparison, data obtained in [a si]milar way from 15 normotensive rab[bits] (Thorén and Jones 1977).

[af]ferents from very high threshold baroreceptors as have been described using an *in vitro* [ao]rtic arch preparation in normotensive rats (Thorén *et al.* 1977).

The mean arterial blood pressure level at which the C-fibres either started firing or at [w]hich sporadic afferent traffic became synchronous with the cardiac cycle is shown for each [fi]bre in each animal in Fig. 1. Similarly the threshold values for the medullated fibres are [sh]own in Fig. 1. All of the medullated and 37 of the C-fibres have threshold values lower [th]an the awake blood pressure of the animal, *i.e.* the great majority of the C-fibres would [ha]ve been firing under normal circumstances in these hypertensive animals. The mean thresh[o]ld for the medullated fibres is 109 ± 2 mmHg while that for the C-fibres is 122 ± 4 mmHg. [T]here is considerable overlapping in the thresholds of the medullated and the non-medullated [fi]bres, which was not the case in the normotensive group (Fig. 2).

The combined data on the thresholds for the medullated and the non-medullated fibres [are] shown in Fig. 2. For comparison the threshold data obtained in a similar way in normo[tens]ive rabbits (Thorén and Jones 1977) are shown in the upper part of the figure. At the [aw]ake mean blood pressure of the normotensive group 29 out of 32 (91 %) medullated and [11 o]ut of 40 (28 %) of the C-fibres are firing. In the hypertensive group the equivalent figures [a]re 54 out of 54 (100 %) medullated fibres firing as are 37 out of 47 (78 %) C-fibres. Although [the] C-fibre receptors are clearly reset in the hypertensive animals they have not reset to the [sa]me extent as the receptors with medullated fibres. The differences in blood pressure when [50 %] of both fibre groups have reached threshold are 30 mmHg for the normotensive animals [an]d only 15 mmHg for the hypertensive rabbits (Fig. 2).

The receptor response to lowering the aortic arch pressure by caval vein occlusion [an]d to raising the pressure by descending aortic occlusion was determined for 19 C-fibres

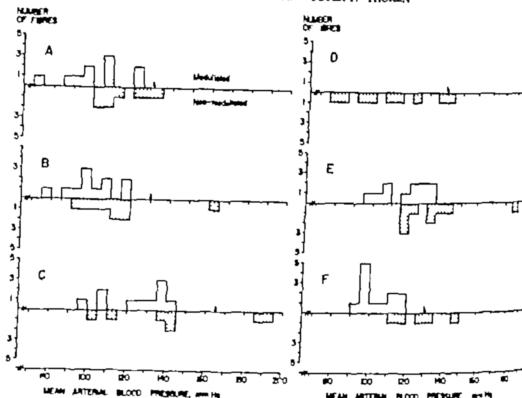


Fig. 1 The thresholds for activation of 54 medullated and 47 non-medullated fibres are shown. Each animal is plotted separately. The arrows indicate the awake mean arterial blood pressure in the individual animal.

dissected until only few fibres or in most cases, single fibre preparations remained. All such filaments were then tested by a brief occlusion of the descending thoracic aorta.

The receptors were then localized to the aortic arch region by their lack of response to brief ascending aortic occlusion and by their response to descending aortic occlusion. In some cases they were localized more accurately by gentle probing with a fine probe in which case they were found to be distributed throughout the aortic arch. A similar distribution was previously noted in normotensive rabbits (Thorén and Jones 1977) where the great majority of receptors had been localized by gentle probing.

Changes in aortic pressure were produced by occlusion of either inferior vena cava or descending thoracic aorta and the changes in receptor activity during such manoeuvres recorded.

Results

The awake mean blood pressure of the 6 rabbits prior to induction of hypertension was 85 ± 4 mmHg (mean ± S.E.). At the time of study 6–9 weeks later the mean blood pressure was 143 ± 6 mm (for individual values see Fig. 1). The results described below are based on recordings from 47 C-fibres and 54 medullated fibres from the left aortic nerve. The conduction velocities for the C-fibres varied from 0.4 to 1.7 m/s (mean ± S.E. = 0.91 ± 0.4 m/s).

All receptors studied were localized to the aortic arch region by their response to occlusion of the descending aorta and the lack of response or decrease in activity when the ascending aorta was clamped. As in the normotensive rabbit (Thorén and Jones 1977) the aortic nerve appeared to contain C-fibres from endings that could be localized to the heart (confirmed in many cases by mechanical probing of the myocardium) and C-fibres to which a function could not be ascribed as they did not respond to the maximum pressure changes attainable with this preparation (range 175–220 mmHg). It is possible some of these are

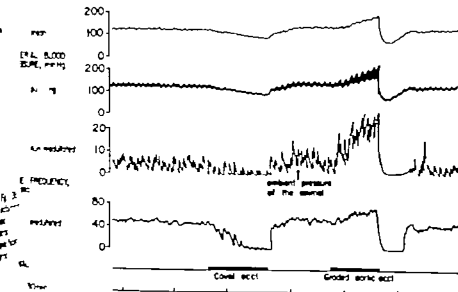


Fig. 4 Effect of graded caval ... occlusion and aortic occlusion on aortic blood pressure and spik... fre... by a one medullated and one non-medullated aortic baroreceptor afferent in the same filament. The ...medullated afferent has ... somewhat higher threshold and lower discharge rate than the medullated ... The mean awake blood pressure (128 mmHg) for this hypertensive rabbit is indicated by an arrow

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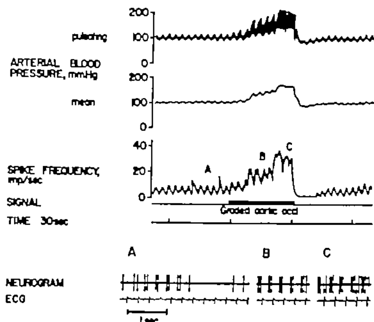


Fig. 3 Effect of a graded aortic occlusion on aortic blood pressure and spike frequency in a single aortic C-fibre. The letters in the spike frequency recordings correspond to the neurogram below

and 12 medullated fibres. Fig. 3 shows the relationship between the discharge frequency and the mean arterial blood pressure in one of these C-fibres during graded aortic occlusion.

Fig. 4 shows the simultaneous discharge rate in one medullated and one C-fibre in the same filament during graded aortic occlusion. During the manoeuvres the different variables were recorded on a Philips analog 7 tape recorder so that they could be replayed and the spike counter first set to record the C fibre activity and then reset to record the activity of the medullated fibre. The C fibre has a higher threshold than the medullated fibre and smaller increase in discharge frequency with increasing pressure.

The combined data describing the blood pressure discharge relation for the 19 non-medullated and the 12 medullated fibres are shown in Fig. 5 This figure, of course, represents only C-fibres in which it was possible to obtain pressure response curves. The high threshold fibres (Thorén *et al* 1977 Thorén and Jones 1977) may have different response frequencies from those C fibres observed here

Total conduction time was obtained for 3 receptors and the firing in relation to the cardiac cycle could then be determined Activation of the receptors were then found to occur in early systole in phase with activation of the medullated fibres. Thus they are similar to the C-fibre traffic in normotensive rabbits (Thorén and Jones 1977)

Discussion

Further evidence for the presence of C-fibres arising from the aortic arch was obtained from this study Such fibres have recently been described in normotensive animals (Fidone and Sa[illegible] 1969 Coleridge *et al* 1973 Thorén *et al* 1977 Thorén and Jones 1977).

Experimental methods In this study the thresholds and pressure response curves of medullated and non-medullated aortic baroreceptors were compared in normotensive and hypertensive rabbit. The results in Fig. 2 suggest that the threshold values for the majority of the

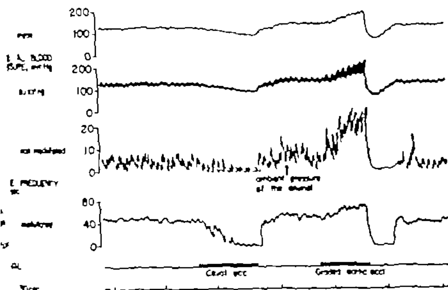


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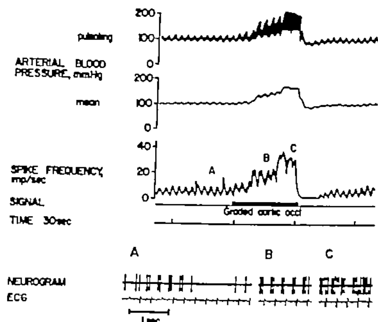


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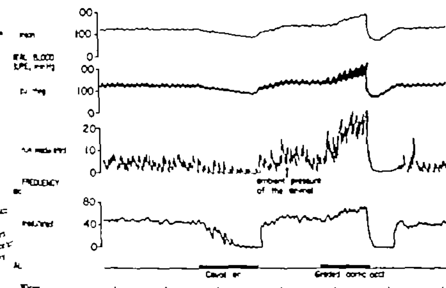


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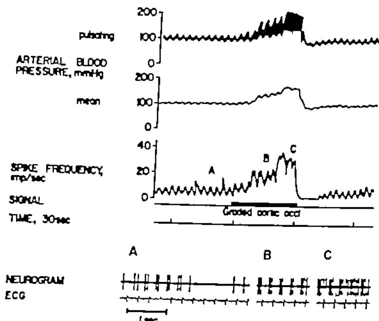


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role of baroreceptor C-fibres in cardiovascular control in hypertension

observation that there is an increased firing in the C-fibres at pressures equal to awake [illegible]d pressure levels makes it likely that the C-fibres in hypertension exert a tonic influence [illegible]he vasomotor centre while in the normotensive animal it appears that they are largely [illegible]hypertensive in nature. For this reason, in hypertensive animals, they may help to [illegible]pensate for blood pressure reductions such as may result *e.g.* from hemorrhage, while [illegible]ormotensive animals they are less effective in this respect since the firing in the C-fibres [illegible]ese animals is sparse under normal conditions.

[illegible] conclusion, therefore, the C-fibres arising from the aortic arch of hypertensive animals [illegible]reset just as the medullated receptors. However the resetting appears to be less complete. [illegible]sequence baroreceptor C-fibres may be of major importance for the tonic, homeo-[illegible]c circulatory control in the hypertensive animal. Reflex studies are required to deter[illegible] the degree of truth in this assertion.

[illegible] work was supported by the Swedish Medical Research Council Project No. 14X-04769 and 14X-00016. [illegible] V. Jones was British MRC travelling fellow for 1975-1976.
[illegible]hanks are due to Mrs Eva-Cari Emanuelson for able technical assistance.

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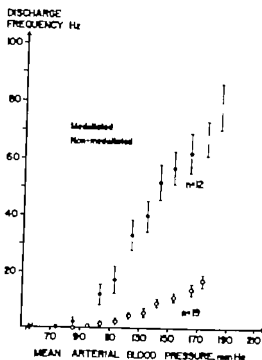


Fig. 5 Pressure response curves for 19 non-medullated and 12 medullated aortic baroreceptor afferents in 6 hypertensive rabbits.

in the same animal. Thus when 50% of the aortic medullated and 50% of the non-medullated fibres are firing the differences in threshold were twice as great in the normotensive in comparison with the hypertensive rabbits. Furthermore, in the hypertensive animals 7[illegible] of the aortic C-fibres are firing at ambient blood pressures in contrast to only 28 [illegible] in the normotensive group of animals as shown by Thorén and Jones (1977). In addition the firing in the C-fibres was 1 1 Hz at the mean ambient pressure of the normotensives (Thorén and Jones 1977) while it was 9 0 Hz at the equivalent pressure in the hypertensives. Thus under normal awake conditions there is a greatly increased impulse traffic from C-fibre receptors in the hypertensive animals compared to the normotensive controls, implying that they exert a more pronounced effect on higher vasomotor centres in the former It is worth noting that also the mean traffic in the 12 medullated fibres examined in the hypertensive animals was higher at ambient pressure than the traffic in 12 medullated fibres in the normotensives (Thorén and Jones 1977) Thus the mean firing at awake pressures in the normotensives was 19 Hz in contrast to 48 Hz in the hypertensives.

Why do the C-fibres not reset as much as the medullated fibres?

Resetting of the medullated baroreceptors has been postulated to be due to either altered distensibility of the receptor area (Aars 1968) to degeneration of the baroreceptor nerve endings (Abraham 1967 Hilgenberg 1967) or to a combination of the two (Angell James 1973). If altered distensibility of the aortic arch is responsible for resetting, and it seems likely that this is at least partly the case since reversal of aortic baroreceptor resetting has been shown to occur within a matter of hours after abolition of the hypertension (Salgado and Krieger 1973), then the different degree of resetting seen with the C fibre receptors implies that they are linked up to the wall of the aorta in a different manner or at a different site from the medullated fibre receptors.

action of the catecholamine with the β-receptor The sequence of events leading from interaction between the β-adrenoceptor and an agonist to a response has been partic ly ell characterized in the adipose tissue (*cf* Fain 1973). We have therefore used this e to study the effects of acidosis *in vivo* and *in vitro*. We recently showed that acidosis bits cyclic AMP formation in isolated rat fat cells (Fredholm and Hjemdahl 1976). ever lipolysis at decreased pH did not reach control levels even if fat cyclic AMP eased to supramaximal levels (Hjemdahl and Fredholm 1976 a). This suggests that osis inhibits lipolysis also at a step subsequent to cyclic AMP formation.

is possible that the antilipolytic effect of acidosis is mediated by an endogenous inhibitor polysis. At present there are three main candidates for a role as endogenous inhibitor ipolysis: adenosine (Schwabe *et al.* 1975), fatty acids (Rodbell 1965 Fain and Shepherd 5) and the so-called feed-back regulator of Ho and Sutherland (1975). It is also possible t pH changes could induce changes in the cellular cation composition (Brown and Goott d, Carvalho *et al* 1963), which may influence lipolysis (Mosinger 1970)

An antilipolytic effect of acidosis mediated over the accumulation of an endogenous ibitor or a change in ionic composition may be considered an "indirect" effect of acidosis. purpose of the present report is to examine whether a decrease in pH is antilipolytic virtue of such an indirect mechanism of action or whether the inhibitory effect can best explained on the basis of a direct effect of the hydrogen ion. We ha therefore examined influence of modifications of the incubation media. Furthermore, the effect of decreased I on lipolysis in "perifused" fat cells, as described by Allen and co-workers (1973 1976) examined The latter technique has two advantages firstly it reduces the influence of accumulated material(s) in the medium surrounding the fat cells and secondly it allows a netic analysis of activation and inhibition of lipolysis.

Materials and Methods

Epididymal fat pads from 3–5 Sprague-Dawley rats (Anticimex-strain), weighing 180–220 g, were pooled each expt. Isolated fat cells ere prepared as described by Rodbell (1964), using incubation ith 3 mg/ml ude bacterial collagenase (Worthington) for 40–60 min.

Incubation experiments The fat cells were counted in Bürker chamber and diluted to final concentration of 50–70 000 cells/ml in Krebs-Ringer phosphate buffer containing, unless otherwise stated, 5 5 mM ucose, 1 1 mM $CaCl_2$ and 3 albumin (Bovine fraction V Sigma, St. Louis). When the ionic composition altered the NaCl concentration was adjusted to give constant ionic strength The cells were preincubated at 37°C, usually for 15 min, before the addition of lipolytic drugs

Perifusion experiments. The expts with "perifused" fat cells were conducted essentially as described by llen and co-workers (1973). Thus, 1 ml of packed fat cells ere placed in temperature (37°C) controlled, ater jacketed chamber containing 2 ml buffer and were perifused at the rate of 2 ml/min, with Krebs-Ringer phosphate buffer of indicated pH containing 1.8 mM $CaCl_2$, 5.5 mM glucose and 1 albumin. Lipolysis was stimulated by the infusion of noradrenaline (as the hydrochloride, Sigma, St. Louis), diluted buffer containing 20 μg/ml ascorbic acid, via side arm in the perifusion system. In some expts.)-7 ^{3}H-noradrenaline hydrochloride (7 5 Ci/mmol New England Nuclear Chicago) was added in race amounts in order to determine the shape of the noradrenaline concentration profile. Our results in his respect agree completely ith those of Allen *et al.* (1973, 1976)

Assays. Lipolysis was determined as glycerol production (in nmoles/unit time) and in some expts. fatty acids were also determined by titrimetric method (Trout *et al* 1960) In the incubation expts. glycerol was measured exactly as described by Laurell and Tibbling (1966). I the perifusion expts the initial deproteinization step as omitted. Standard curves ere therefore prepared using albumin-containing

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Abstract

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The possibility that acidosis inhibits lipolysis indirectly by causing ionic shifts or by favouring the accumulation of an inhibitor has been tested in isolated fat cells. Lipolysis induced by 3 μM noradrenaline (NA) was inhibited by 40-60 % and that induced by 1 mM theophylline (THEO) by about 75 % when the pH was reduced to 6.6. Lipolysis induced by NA + THEO was inhibited by 20-30 %. Changing the concentration of Ca^{++} or Mg^{++} did not alter the degree of inhibition. Reducing the K-ion concentration enhanced the inhibitory effect of low pH on lipolysis induced by NA or NA + THEO, whereas cyclic AMP accumulation was uninfluenced. Omitting glucose from the incubation medium caused a slight enhancement of pH induced inhibition of lipolysis (from 60 to 70 %, $p < 0.01$). Reducing the concentration of albumin, which binds inhibitory substances such as FFA, reduced lipolysis more at normal than at reduced pH. At high FFA/albumin ratios (5 or above) lipolysis was similar at normal and reduced pH. The antilipolytic effect of decreased pH was equally pronounced in perfused fat cells, where inhibitory substances are not allowed to accumulate. Our results suggest that the antilipolytic effect of acidosis is mainly a direct effect of the increase in H-ion concentration. The inhibitory effect of acidosis on various responses to β-adrenoceptor stimulation may be caused by a decreased formation of cyclic AMP in turn caused directly by the decrease in pH

Changes in pH influence the responses to catecholamines and to sympathetic nerve stimulation There seems to be general agreement that acidosis counteracts those effects of catecholamines that are mediated over β-adrenoceptors. Thus it has been shown that adrenergic β-effects in the heart (Mitchell *et al* 1972, Shaer 1974), bronchial (Blumenthal *et al* 19[illegible]) and vascular (Wagner *et al* 1975) smooth muscle are counteracted by decreased pH. Furthermore, metabolic effects of β-adrenergic stimulation are inhibited by acidosis (Poyart and Nahas 1966, Reynolds and Haugaard 1967)

It is unlikely that the inhibition of the catecholamine effect by reducing pH from 7.4 to 6.6 is due simply to a change in the degree of ionization of the amine (Reynolds and Haugaard 1967) It is also unlikely that the effect is specifically located to the catecholamine receptor since lipolysis induced by ACTH is inhibited to a similar degree as that induced by noradrenaline (Wenkeová and Mosinger 1965 Poyart and Nahas 1968). Thus, the inhibition of adrenergic β-effects by acidosis is probably due to inhibition of a step beyond the

[illegible]action of the catecholamine with the β-receptor The sequence of events leading from [illegible]eraction between the β-adrenoceptor and an agonist to a response has been partic[illegible]y well characterized in the adipose tissue (*cf* Fain 1973). We have therefore used this [illegible] to study the effects of acidosis *in vivo* and *in vitro* We recently showed that acidosis [illegible]its cyclic AMP formation in isolated rat f t cells (Fredholm and Hjemdahl 1976). [illegible]ver lipolysis at decreased pH did not reach control levels even if fat cyclic AMP [illegible]imized to supramaximal levels (Hjemdahl and Fredholm 1976 a). This suggests that [illegible] inhibits lipolysis also at a step subsequent to cyclic AMP formation.

[illegible]s possible that the antilipolytic effect of acidosis is mediated by an endogenous inhibitor [illegible] polysis. At present there are three main candidates for a role as endogenous inhibitor [illegible]polysis, adenosine (Schwabe *et al* 1975), fatty acids (Rodbell 1965 Fain and Shepherd [illegible]) and the so-called feed-back regulator of Ho and Sutherland (1975). It is also possible [illegible] pH changes could induce changes in the cellular cation composition (Brown and Goott [illegible], Carvalho *et al.* 1963), which may influence lipolysis (Mosinger 1970).

[illegible] antilipolytic effect of acidosis mediated over the accumulation of an endogenous [illegible]bitor or a change in ionic composition may be considered an "indirect" effect of acidosis. [illegible] purpose of the present report is to examine whether a decrease in pH is antilipolytic [illegible] virtue of such an indirect mechanism of action or whether the inhibitory effect can best [illegible] explained on the basis of direct effect of the hydrogen ion. We have therefore examined [illegible] influence of modifications of the incubation media. Furthermore, the effect of decreased [illegible] on lipolysis in "perifused" fat cells, as described by Allen and coworkers (1973 1976) [illegible] examined. The latter technique has two advantages, firstly it reduces the influence of [illegible]mulated material( ) in the medium surrounding the fat cells and secondly it allows a [illegible]etic analysis of activation and inhibition of lipolysis.

Materials and Methods

[illegible]ididymal fat pads from 3–5 Sprague-Dawley rats (Anticimex-strain), weighing 180–220 g, were pooled [illegible] each expt. Isolated fat cells were prepared as described by Rodbell (1964), using incubation with 3 mg/ml [illegible] bacterial collagenase (Worthington) for 40–60 min.

Incubation experiments. The fat cells [illegible] counted in Bürker chamber and diluted to final concentration of 50–70 000 cells/ml in Krebs-Ringer phosphate buffer containing, unless otherwise stated, 5.5 mM [illegible], 1.8 mM $CaCl_2$ and 3 [illegible] albumin (Bovine fraction V Sigma, St. Louis). When the ionic composition [illegible] altered the NaCl concentration [illegible] adjusted to give constant ionic strength. The cells were preincubated at 37 C, usually for 15 min, before the addition of lipolytic drugs

Perifusion experiments. The expts [illegible] "perifused" fat cells were conducted essentially as described by [illegible] and coworkers (1973). Thus, 1 ml of packed fat cells were placed in temperature (37°C) controlled, [illegible] jacketed chamber containing 2 ml buffer and were perifused at the rate of 2 ml/min, [illegible] Krebs-Ringer phosphate buffer of indicated pH containing 1.8 mM $CaCl_2$, 5.5 mM glucose and 1 albumin. [illegible]olysis was stimulated by the infusion of noradrenaline (as the hydrochloride, Sigma, St Louis), diluted [illegible] buffer containing 20 µg/ml ascorbic acid, via side arm in the perifusion system. In some expts. [illegible]-7-^{3}H-noradrenaline hydrochloride (7.5 Ci/mmol New England Nuclear Chicago) was added in [illegible] amounts in order to determine the shape of the noradrenaline concentration profile Our results in [illegible] respect agree completely with those of Allen *et al* (1973 1976).

[illegible] *Assays.* Lipolysis was determined as glycerol production ([illegible] µmoles/unit time) and in some expts. fatty [illegible] acids were also determined by [illegible] titrimetric method (Trout *et al* 1960) In the incubation expts. glycerol [illegible] was measured exactly as described by Laurell and Tibbling (1966). I[illegible] the perifusion expts the initial [illegible] deproteinization step was omitted. Standard curves [illegible] are therefore prepared using albumin-containing

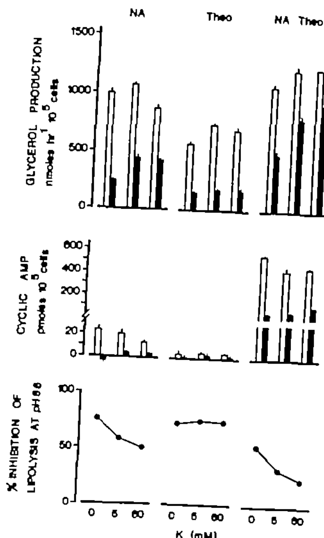


Fig. 1 Glycerol production (during 60 min) and net accumulation of cyclic AMP (at 5 min) in fat cells incubated with 3 μM noradrenaline (NA), 1 mM theophylline (Theo) or the combination of these drugs at pH 7.4 (open bars) and pH 6.6 (filled bars). The cells (50 000/ml) were incubated in buffer containing either no, normal (5 mM) or high (60 mM) K with corresponding adjustment of the Na concentration. A preincubation time of 20–30 min was allowed before the addition of a drug. The number of observations with each treatment is indicated along the columns denoting glycerol production. The lowest panel shows the percental inhibition produced by decreased pH. Note that K influences the antilipolytic effect of decreased pH in NA-stimulated fat cells but not in Theo-stimulated cells.

media in these expts. No significant interference was, however, detected. Cyclic AMP was determined by the competitive binding assay of Brown *et al* (1971) as described previously (Fredholm and Hjemdahl 1976).

Results

Stimulated glycerol production and cyclic AMP formation was inhibited by acidosis as described earlier (Fredholm and Hjemdahl 1976, Hjemdahl and Fredholm 1976a). Thus noradrenaline (3 μM)-stimulated lipolysis was inhibited by 50–60% at pH 6.6. Theophylline (1 mM)-stimulated lipolysis was inhibited by about 75% and lipolysis stimulated by the combination of these drugs by 20–30% (Fig. 1).

Effects of changes in the cationic composition. The results from our expts. where the potassium concentration was either 0, 5 or 60 mM are summarized in Fig. 1. Omission of K decreased lipolysis in all situations tested. In agreement with earlier findings (Mosinger 1970). In contrast, cyclic AMP accumulation was not significantly decreased when potassium was omitted. The inhibition of lipolysis by decreased pH was enhanced when potassium was omitted from the incubation medium, except when theophylline alone was used as the

[illegible] Inhibition of glycerol release and cyclic AMP accumulation in isolated rat fat cells by acidosis (pH 6.6) in the absence and in the presence of glucose (5.5 mM). The values are mean ± S.E. of quadruplicate incubations.

Glucose		Lipolysis at pH 6.6 (% of control)	cAMP accumulation at pH 6.6 (% of control)
[illegible]		26±1	36±13
[illegible])	+	33±2	22±5
		p 0.05	
		31±2	44±17
[illegible])	+	42±2	30±8
		p 0.01	

[illegible]ulating agent. The inhibition due to decreased pH was significantly less pronounced [illegible] 0.01) in high K -buffer when noradrenaline or noradrenaline + theophylline were [illegible] as stimulating agents.

[illegible] 10-fold increase in the magnesium ion concentration (from 1.2 to 12 mM) reduced [illegible]adrenaline (3 μM)-stimulated lipolysis by about 10%, but did not alter the cyclic AMP [illegible]mulation. The inhibition of lipolysis by decreased pH was unaffected by the change in [illegible] concentration. By omission of Ca and the addition of 1 mM EGTA nor[illegible]aline (3 μM)-stimulated lipolysis was inhibited by approximately 10% (590 ± 30 [illegible] 550 ± 30 nmol h 10^{-4} cells n 5). The antilipolytic effect of pH reduction was not [illegible]enced by the omission of calcium ions (53% inhibition with Ca ++ 50% inhibition [illegible]hout).

[illegible] Effect of altered glucose and albumin concentration. Since it has been suggested that glucose [illegible] counteract the antilipolytic effect of acidosis (Poyart *et al* 1967) expts. were carried [illegible] in the presence and absence of glucose. As seen in Table I our data confirm the previous [illegible]ervation, but the effect is minor. The inhibition of cyclic AMP accumulation was not [illegible]enced by omitting glucose (p 0.02, one-tailed t-test).

It has also been reported that excess albumin could reduce the antilipolytic effect of [illegible]dosis (Poyart *et al* 1967). We could not confirm this finding. On the contrary as shown [illegible] Fig. 2, decreasing the pH to 6.6 produced more marked inhibition of noradrenaline [illegible]μM)-stimulated lipolysis when the FFA/albumin-ratio was kept low by the addition of [illegible] albumin. There was a significant inverse relationship (r −0.84 n 18) between [illegible] rate of lipolysis and the FFA/albumin ratio at a normal pH. At pH 6.6 the reduction in [illegible]olysis with increasing the FFA/albumin ratio was significantly (p 0.01 analysis of [illegible]ariance) less pronounced. This obviously suggests that inhibition of lipolysis by acidosis [illegible] by increasing the FFA/albumin ratio are two independent processes.

Perfused fat cells. The inhibition of NA-stimulated lipolysis by acidosis was studied in [illegible] expts. with perfused fat cells (Table II). As shown previously in incubated fat cells [illegible]ckholm and Hjemdahl 1976) the inhibition was more pronounced the lower the pH [illegible] at apparently similar with varying concentration of noradrenaline (Table II). I most [illegible] expts. the effect of acidosis was assessed by comparing the lipolytic effect of noradrenaline [illegible] two aliquots of fat cells, one perfused with buffer pH 7.4 one with buffer of a lower [illegible] pH. The noradrenaline concentration in the medium rose and fell with a half-time of less

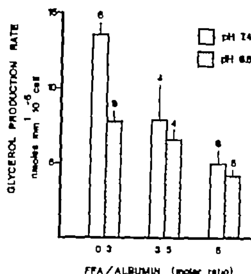


Fig. 2. The influence of FFA accumulation [illegible] glycerol production rate in isolated fat cells [illegible] incubated with 3 μM noradrenaline at normal [illegible] reduced pH. The albumin concentration of the [illegible] tion buffer varied between 0.5 and 5 [illegible]. The molar [illegible] of FFA/albumin in incubates containing various [illegible] concentrations were determined at 40 and [illegible] incubation with noradrenaline. The glycerol production rate during the 40 min periods of incubation [illegible] these determinations is shown in incubates [illegible] (0–3), intermediate (3–5) or high (more than 5) FFA/albumin ratios. The number of observations [illegible] are indicated in the figure. Note that accumulation [illegible] FFA (i.e. a high FFA/albumin ratio) has [illegible] nounced inhibitory effect at normal than at decreased [illegible]

than 2 min both at pH 7.4 and pH 6.6 (not shown). Glycerol production rose much [illegible] slowly, reaching a plateau after 15–25 min both at normal and reduced pH. After cessation of noradrenaline infusion the rate of glycerol production returned towards normal [illegible] half time of 5–7 min at normal and reduced pH. These results are similar to those reported earlier for noradrenaline-stimulation in fat cells perifused with Krebs-Ringer bicarbonate buffer pH 7.4 without glucose (Allen *et al.* 1973, 1976).

In 3 other expts. the pH of the perifusing buffer was changed while the cells were exposed to noradrenaline. One expt. of this type is illustrated in Fig. 3. It is seen that the change [illegible] pH made the cells approach a new steady level of lipolysis, which was reached after 1[illegible] min.

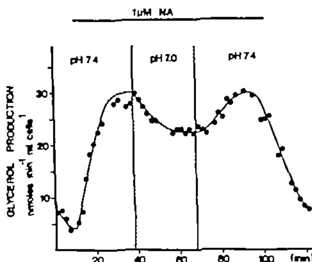
Discussion

Acidosis inhibits lipolysis in 3 experimental situations: incubated fat cells, perifused cells and *in situ* perfused dog adipose tissue. These preparations should differ mark[illegible] with respect to the accumulation of inhibitory substances. As seen in Table III acid[illegible] inhibits lipolysis to a similar degree in these different preparations. This may be take[illegible]

TABLE II. Net lipolytic responses to noradrenaline of fat cells (1 ml) perifused with [illegible] ml buffer [illegible] 7.4 (control) and lower pH

pH	Noradrenaline μM	Glycerol production (moles min ml cells^{-1}) Control	Low pH	Inhibition
7.0	0.3	35	21	39
7.0	1.0	45 ± 9[a]	30 ± 4	31 ± 9[a]
7.0	10	38 ± 10[b]	20 ± 6[b]	42 ± 15[b]
6.8	1.0	7	1	56
6.6	1.0	45	15	67
6.6	1.0	23	4	81

[a] Mean values ± S.E. from 4 observations, 3 of which were of the type illustrated in Fig. 3.
[b] Mean values ± S.E. from 4 paired observations.



...3 Glycerol production rate in ...ted fat cells (1 ml packed ...) perifused with ... ml buffer/... Lipolysis was stimulated ... 1 µM NA during 90 min, as ...cated in the figure. Note that ...es min to change from one steady ... to another approximately ...0 min both after induction of ...lysis and ... change in pH ...ng NA stimulation

...first argument against the effect of acidosis being mediated over an accumulating in...itor. The second argument is derived from an examination of the kinetics in the perifused ...ell system. After noradrenaline infusion lipolysis returned towards basal rates with ...alf-life of 5-7 min irrespective of whether the cells were perifused with buffer at pH ... 7 0 or 6.6. This implies that acidosis does not change the rate-limiting step in the in...hibition of lipolysis. Furthermore, the time necessary to go from one steady-state to ...other was the same whether the transition was from basal to stimulated at pH 7 4 7 0 ... 6 6 or from stimulated pH 7 4 to stimulated pH 7 0 and *vice versa* (*e.g.* Fig. 3). These ...dings are not easily reconciled with an indirect mechanism of action of acidosis. Thirdly ... have examined more directly the importance of different possible mediators of the anti...lipolytic effect of acidosis.

...Modifications of the calcium or magnesium ion concentrations in the medium had no ...ffect on the antilipolytic effect of acidosis, even though some effect on the magnitude of the ...sponse to noradrenaline was seen. Changes in the distribution of these ions, which may ...ccur during acidosis (Carvalho *et al.* 1963), therefore seem to have negligible influence ...n the antilipolytic effect of acidosis.

There is, however, evidence that K⁺-ions may somehow be involved in the inhibitory ...ffect of acidosis. Decreased pH may cause a shift of potassium out of cells (Brown and ...Goott 1963). We found that an increased potassium ion concentration counteracted the ...antilipolytic effect of acidosis when NA was used as the stimulating agent. K⁺-ions appear ...to influence ... step beyond the formation of cyclic AMP since lipolysis was depressed by ...reducing potassium, without significant effect on cyclic AMP. Furthermore, the effect ...of K⁺ on lipolysis was also seen when NA and theophylline were combined as lipolytic ...agents. Since this combination gives cyclic AMP levels in the fat cells that are supramaximal ...with respect to lipolysis (Hjemdahl and Fredholm 1976 a), this opens the possibility that the ...less pronounced cyclic AMP-independent inhibition of lipolysis is caused by a decrease in ... the intracellular K⁺-ion concentration.

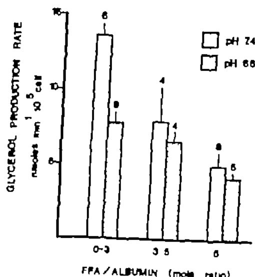


Fig. 2. The influence of FFA accumulation on glycerol production rate in isolated fat cells incubated with 3 μM noradrenaline at normal and reduced pH. The albumin concentration of the incubation buffer varied between 0.5 and 5 %. The molar ratio of FFA/albumin in incubates containing various concentrations were determined at 40 and 80 min incubation with noradrenaline. The glycerol production rate during the 40 min periods of incubation between these determinations is shown in incubates with low (0–3), intermediate (3–5) or high (more than 5) FFA/albumin ratios. The number of observations are indicated in the figure. Note that accumulation of FFA (i.e. a high FFA/albumin ratio) has a more pronounced inhibitory effect at normal than at decreased

than 2 min both at pH 7.4 and pH 6.6 (not shown). Glycerol production rose much more slowly reaching a plateau after 15–25 min both at normal and reduced pH. After cessation of noradrenaline infusion the rate of glycerol production returned towards normal with a half time of 5–7 min at normal and reduced pH. These results are similar to those reported earlier for noradrenaline-stimulation in fat cells perfused with Krebs Ringer bicarbonate buffer pH 7.4 without glucose (Allen *et al.* 1973, 1976).

In 3 other expts. the pH of the perifusing buffer was changed while the cells were exposed to noradrenaline. One expt. of this type is illustrated in Fig. 3. It is seen that the change in pH made the cells approach a new steady level of lipolysis, which was reached after 15 min.

Discussion

Acidosis inhibits lipolysis in 3 experimental situations: incubated fat cells, perifused cells and *in situ* perfused dog adipose tissue. These preparations should differ markedly with respect to the accumulation of inhibitory substances. As seen in Table III acidosis inhibits lipolysis to a similar degree in these different preparations. This may be taken

TABLE II. Net lipolytic responses to noradrenaline of fat cells (1 ml) perifused with 2 ml buffer/min at pH 7.4 (control) and lower pH

pH	Noradrenaline μM	Glycerol production (μmoles min⁻¹ ml cells⁻¹)		% inhibition
		Control	Low pH	
7.0	0.3	35	1	39
7.0	1.0	45±9[a]	30±4	31±9[a]
7.0	10	38±10[b]	20±6[b]	42±15[b]
6.8	1.0	27	12	56
6.6	1.0	45	15	67
6.6	1.0	23	4	81

[a] Mean values±S.E. from 4 observations, 3 of which were of the type illustrated in Fig. 3.
[b] Mean values±S.E. from 4 paired observations.

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TABLE III Inhibition of NA-induced lipolysis *in vitro* and *in vivo* by decreased pH

	pH 7.0 (% of response at pH 7.4)	Ref.
Isolated fat cells (3 μM NA)	(45)[a]	[b]
Perifused fat cells (10 μM NA)	4	Table II
I v NA infusion (0.35 μg h⁻¹ min⁻¹)—constant flow perfused canine adipose tissue	56	
Sympathetic nerve stimulation (4 Hz)—free flow perfused canine adipose tissue	40	[d]

[a] Since the pH is not stable during conventional incubation of fat cells this figure was obtained b[y ex]trapolation.
[b] Fredholm and Hjemdahl 1976
[c] Hjemdahl and Fredholm 1976 b.
[d] Hjemdahl 1976.

Adenosine is known to inhibit cyclic AMP accumulation in fat cells while lipolysis i[s] inhibited to a minor degree, if at all (*e.g.* Hjemdahl and Fredholm 1976, Schwabe *et al* 1975) Acidosis, on the other hand, inhibits lipolysis at least in parallel with inhibition of cyclic AMP accumulation (Fredholm and Hjemdahl 1976, Hjemdahl and Fredholm 1976 b) Thus, it is unlikely that the antilipolytic effect of acidosis is due to adenosine. The inhibitory effect of adenosine is, however potentiated by a decreased pH (Hjemdahl and Fredholm 1976 a) and the two might act synergistically

Both free fatty acids (Rodbell 1965) and the feed-back regulator of Ho and Sutherland (1975) are strongly bound to and antagonized by albumin Therefore our finding that the antilipolytic effect of acidosis was enhanced rather than depressed by increasing the albumin concentration in the medium (Fig. 2) argues against the opinion that either of these in-hibitors mediate the effect of acidosis. Furthermore direct measurements of cell-associated fatty acids showed that their level was lower at reduced than at normal pH (Fredholm and Hjemdahl 1976) Thus, there is no good evidence that either of these proposed feed-back inhibitors mediate the effect of acidosis.

We feel it is justified to conclude from these studies that the effect of acidosis on lipoly[sis] is not to any major degree mediated over the accumulation of any endogenous inhibitor or to transcellular shifts of potassium, magnesium or calcium. This finding may be relevant for our understanding of how acidosis inhibits also other β-adrenoreceptor responses. [In] particular the possibility must be considered that acidosis causes an inhibition of cyclic AMP formation in most tissues, just as it does in fat cells and that this provides an explana-tion for the antagonism of β-adrenoreceptor responses thought to be mediated over cyclic AMP

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ch can give rise to erroneous estimations of the quaternary amines. Naturally those inter g factors that are recognized and understood can mostly be eliminated by appropriate sures, but it is still uncertain whether other unpredicted factors are present or not. An r important disadvantage of bioassay methods is that they are often very laborious and -consuming.

recent years great progress has been made in the development of chemical methods the determination of choline and acetylcholine. The radioenzymatic methods (Saelens, ta and Simke 1970, Reid, Haubrich and Krishna 1971 Goldberg and McCaman 1973) the gaschromatographic-massspectrometric methods (Jenden 1973 Karlén *et al.* 1974) ld be mentioned. It was, however felt that the measurement of acetylcholine released intestine would be greatly aided by simpler or more inexpensive methods.

approach in which nerve-containing preparations are incubated with radioactively lled choline in order to mark the transmitter stores of the nerves with labelled acetyline has been used in metabolic studies of the rat diaphragm (Saelens and Stoll 1964 rr 1970), the cat heart (Wallach, Goldberg and Shideman 1967), the cat superior cervical glion (Friesen, Kemp and Woodbury 1965, Colber and Lang 1969 Collier and MacIntosh 9) and the rat cerebral cortex (Browning and Schulman 1968, Richardson and Szerb 4). More recently this technique has also been applied in the guinea pig ileum (Szerb 75, 1976).

he present study was undertaken with the intention of investigating if the use of radiove tracers could substitute for other methods in the measurement of acetylcholine re ed from guinea pig ileum. The preparation was incubated with H-choline of high specific tivity whereafter the release of ^{3}H-acetylcholine could be studied.

Methods

h guinea pigs weighing 400–550 g were killed by blow on the neck and exsanguinated. A 10 cm long of the intestine was removed, beginning 10 cm proximal to the ileocaecal sphincter and freed from tery by dissection. The longitudinal muscle ith its adherent nerve plexus was prepared as described viously (Wikberg 1976) Preparation weighing about 40–60 mg ere mounted in water jacketed n chamber ith capacity of 4 ml, containing Krebs solution of the following composition (m): NaCl 122 8, KCl 4 7 MgCl 1 2, $CaCl_2$ ..5, $NaHCO_3$ 15 4, KH_2PO 1 2, glucose 5 5 and eserine 0 03. buffer as equilibrated at 37°C by gas mixture of 95 O_2 and 5% CO_2. The preparation was given tial tension of 5 10^{-3} N, the tension being recorded isometrically ith Grass FT 03 transducer Grass polygraph. The organ bath had two 20 l mm platinum electrodes mounted in its all, 10 mm t, parallel to the interposed preparation

The preparation was incubated with 20 μCi ^{3}H-choline (methyl ^{3}H-choline chloride 10.1 Ci/mmol, diochemical Centre, Amersham, England) at final concentration of 1 10^{-6} M, for 1 h It as then ed briefly in the Krebs solution, hereafter 4 ml fractions were collected in 5 min periods for subquent analysis. In some expts the preparation as stimulated electrically by 30–1 000 rectangular pulses h duration of 1 ms and frequency of 0 1–10 Hz, generated by Grass SD9 stimulator. The stimulation s checked ith Tektronix 5103N oscilloscope and the pulses delivered caused current of 320 mA at –16 V. This stimulation has previously been found to be supramaximal ith regard to the motor ch response of the longitudinal guinea pig ileum (Wikberg 1976). After completion of these procedures preparation was decomposed and dissolved on 0 5 ml Soluene 350 (Packard Instrument Company Inc.), d counted in 15 ml of toluene-based scintillation cocktail.

Biochemical assay

he fractions were immediately acidified by the addition of 25 μl of 2 N HCl to bring the pH to about 4–5 nd cooled in an ice bath. From the samples aliquots of 0 5 ml were taken and counted in 10 ml

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Release of ^{3}H-Acetylcholine from Isolated Guinea Pig Ileum. A Radiochemical Method for Studying the Release of the Cholinergic Neurotransmitter in the Intestine

By

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Abstract

WIKBERG J *Release of ^{3}H-acetylcholine from isolated guinea pig ileum. A radiochemical method for studying the release of the cholinergic neurotransmitter in the intestine* Acta physiol scand 1977 *101* 302–317

Innervated strips of longitudinal muscle of guinea pig ileum were incubated with ^{3}H-choline in the presence of physostigmine after which the efflux of tritiated metabolites was studied. Under resting conditions ^{3}H-acetylcholine, ^{3}H-choline and other tritiated metabolites were released into the incubation media. Analysis of the kinetics of the release of the metabolites indicated that the release occurred from at least 2 different compartments. Under the unstimulated conditions used, between 7? and 78 of the radioactivity remained in a third non-releasable compartment. The release of ^{3}H-acetylcholine showed spontaneous fluctuations which were positively correlated to spontaneous variations of the isometric tension of the preparation Electrical field stimulation at low frequency (0 5 Hz) consistently increased the release of ^{3}H-acetylcholine. The release of ^{3}H-choline was increased only slightly or not at all by the electrical stimulation. For studying stimulation-evoked release of ^{3}H-acetylcholine it is suggested that the measurement of the total quaternary amine release is of a similar value to measurement of ^{3}H-acetylcholine. This approach considerably simplified the method During 5 consecutive stimulation periods of 1?0 pulses each, the increase in the release of total quaternary amines diminished with time. By taking into account the reduction of the stores of radioactivity in the tissue during the experiment, correction for the negative trend could be made When the preparation was stimulated with between 30 and 3 000 pulses a close correlation was found between the increase in the release of total quaternary amines and the number of pulses delivered. The volley output of the quaternary amines was not constant, however but diminished by increasing stimulation frequency

Key words: Acetylcholine release ileum, isotope labelling

In studies of cholinergic mechanisms, the isolated mammalian intestine has often been utilized among other experimental systems. When for different reasons, acetylcholine has been measured, a biological assay method has generally been chosen. Choline can also be determined after acetylation to acetylcholine (Hunt 1915) The biological methods are sufficiently sensitive for most applications but some problems are encountered. The precision and specificity of these methods can be questioned. The assays may be influenced by a large number of factors, such as the presence of drugs o active substanc

$$f(t, A, a) = \sum (e^{a_i \Delta t} - 1) A \quad e^{a_i \Delta t} \tag{2}$$

...uld be noted that the non-releasable store, B, does not appear in equation (2).

...ne of the experimental data ... are fitted to these equations by non-linear least square regression. This ...chieved through linearizations of the models by Taylor series expansions and solving for the constants ...ltiple linear regression in an iterative algorithm. Draper and Smith (1966) give the outline of the math... ...eneral terms. For this purpose ... general regression programme ... as written in BASIC, ...hich had ...imum capacity of estimating 20 constants of an arbitrary function.

...he release of radioactivity to the fractions is summed from t ... 0 to $\to \infty$, then ... has

$$\lim_{n \to \infty} \sum \sum (e^{a_i \Delta t} - 1) A \quad e^{a_i \Delta t} = \sum A \tag{3}$$

... the sum of all A represents the total amount of radioactivity that can be released from the prepara-...

... the analysis of the stimulation-evoked release of radioactivity ... method of presenting the data ... as ...ized. The release of total quaternary amines was calculated as

$$\Delta TQA_s = TQA_{at\ stim} - TQA_{pre\text{-}stim}$$

... the unstimulated release of total quaternary amines (TQA) ... as the release just prior to the electrical ...ulation. ΔTQA_s was expressed in per cent of the total amount of radioactivity calculated to be present ...issue at the beginning of the expt. This value could be regarded as uncorrected with respect to the ...l stores of radioactivity that could be released at the moment of stimulation. Correction ... as made by ...pressing the stimulation-evoked release as per cent of the stores of radioactivity (M) calculated to be ...ent in the tissue at the beginning of the stimulation period

$$\Delta TQA = \frac{\Delta TQA}{M} \quad 100$$

...ere M is expressed in per cent of the amount at the beginning of the expt. (M_0). An ...ssentially similar ...thod of correcting the data has previously been applied by others in analyses of ...H-norepinephrine ...ease by electrical stimulation of adrenergically innervated organs (Stjärne and Brundin 1976).

Results

...tification of labelled metabolites released from the unstimulated guinea pig ileum

...er the incubation with ...H-choline, the preparation was washed for 30 min with 6 bath ...ges of Krebs solution, and fractions of the solution were then collected for analysis. ...dioactivity was released spontaneously from the preparation at that time. Direct applica...ion of one ml of the washing solution on the Amberlite column yielded 3 peaks. One peak ...eared between 2.5 and 7.5 ml in the void volume (6.8 ml) of the column, and thus peak ...eluted with water. It will be referred to hereafter as the unbound fraction (UBF). The ...ond peak appeared after 9–14 ml and the third after 15–21 ml of 0.1 M phosphate buffer ...7.0 (Fig. 1 A). These peaks had the same retention ... olumes as native acetylcholine and ...line, respectively. Under resting conditions 24.2 ± 6.9% of the activity appeared in the ...ound fraction and 38.9 ± 4.4% and 36.9 ± 5.8% in the acetylcholine and choline peaks, ...pectively (S.E. of mean, n ... 12). After precipitation of the tissue perfusate with saturated ...monium reineckate as described under Methods (p. 304), and batch treatment with ...l m-equiv. Biorex 9 in 1 ml water ... no activity could be eluted with water after the applica...n of the sample on the Amberlite column. The acetylcholine and choline peaks were still ...ted by the phosphate buffer after this procedure.

or a Triton-based scintillation cocktail. A 2 ml sample was taken for paper chromatography [illegible] To the rest of the sample 0.5 μmol acetylcholine chloride and 0.5 μmol choline chloride were added as carriers. The quaternary ammonium compounds were precipitated by the addition of 0.5 ml of a [illegible] solution of ammonium reineckate. After 1 h at +4°C the sample was centrifuged and the supernatant discarded. The pellet was washed once with 2 ml of a diluted solution of cold ammonium reineckate [illegible] rated ammonium reineckate/water 1 5 (/)) and centrifuged. The pellet [illegible] then batch treated [illegible] m-equiv of Biorex 9 chloride form (Bio Rad Laboratories), in 2 ml ethanol at room temperature [illegible] centrifugation the ethanol was poured into scintillation vials. The ion exchanger [illegible] washed [illegible] with 1 ml ethanol which was added to the scintillation vial. 10 ml of the toluene-based scintillator [illegible] was added to the sample. The recovery of acetylcholine by this method was found to be 97.4±0.5% [illegible] choline 95.9±0.4% (S.E. of mean n = 10 in both cases).

Paper chromatograph To the sample that was to be submitted to paper chromatography 1 μmol [illegible] acetylcholine and 1 μmol choline carrier were added. The sample was precipitated [illegible] reineckate as described above and washed once. The precipitate was then dissolved in 4 ml methanol [illegible] 0.1 m-equiv Biorex and transferred to new tubes. The methanol was evaporated in a stream of [illegible] room temperature, dissolved in 15 μl methanol and applied to Whatman No. 1 filter paper for [illegible] chromatography in a solvent system composed either of acetone/water/10 N HCl 100:20:1 (by vol.) or butane-1-ol/ethanol/water/acetic acid 8 2 3 1 (by volume). The spots were localized by developing [illegible] iodine vapours, cut [illegible] pieces and put into scintillation vials. The radioactivity [illegible] eluted off the paper with 3 ml ethanol/water 1 1 (/v) and counted after the addition of 12 ml Instagel.

Ion exchange chromatography This was used for separating some samples. Amberlite CG 50 [illegible] 400 mesh) was washed with 1 N HCl, with the double volume required to give a negative flame test [illegible] metal ions. It was then washed first with water until the pH exceeded 5.0 and then [illegible] 0.1 M [illegible] phosphate buffer pH 7.0, until the pH reached 7.0. Columns measuring 4 4 450 mm [illegible] packed with [illegible] ion exchanger and equilibrated with the phosphate buffer until the pH of the effluent [illegible] equal to that of the influent. Before the sample was applied the column was washed with 5 ml of water. To one ml of the [illegible] fied tissue perfusate 0.5 μmol choline chloride and 0.5 μmol acetylcholine chloride carrier were added. The sample was applied on the column followed by 5 ml of water and was then eluted at 10 ml [illegible] the phosphate buffer. Fractions of 1.25 ml were collected and counted in 10 ml Instagel. The addition of the carriers was found to markedly increase the resolution of the chromatographic separation.

Liquid scintillation counting The samples were counted in a Packard Tri Carb 3375 liquid scintillation spectrometer in either of three scintillation systems. Water-containing samples were counted in Insta[illegible] (Packard Instrument Company Inc.) or a scintillation cocktail composed of 333 ml Triton X 100, [illegible] toluene, 5 g PPO and 0.1 g POPOP. Solubilized tissue samples and ethanol solutions were counted [illegible] toluene scintillator composed of 1 l toluene, 5 g PPO and 0.3 g POPOP. The quenching was determined by the use of an internal standard.

Synthesis of ^{3}H-acetylcholine

Labelled acetylcholine was synthesized from ^{3}H-choline. Tritiated choline 20 μCi, was added to [illegible] acetic acid anhydride. The mixture was allowed to react for 3 h at room temperature, whereafter the [illegible] acid anhydride was evaporated. The residue was dissolved in a small volume of methanol and applied [illegible] paper for chromatography in the acetone/water/10 N HCl system. The acetylcholine spot was located with a Berthold LB2723 thin layer chromatographic scanner. The spot was cut out and extracted from the paper into methanol.

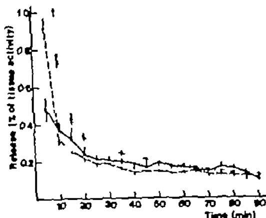
Theory and statistical methods

Let the amount of radioactivity in the tissue at time t be $M(t)$. If it is assumed that the disappearance of radioactivity from the tissue follows a set of n exponential functions, but also that a non-releasable [illegible] (B) of radioactivity is present in the tissue, we have

$$M(t) = \sum_{i} A_i e^{-\alpha_i t} + B$$

where A_i and α_i are constants.

If the radioactivity released was collected in fractions during collection periods of equal duration the activity released during the jth collection period would be given by



2. Washout of radioactivity from the ... guinea pig ileum. The preparation was incubated with ^{3}H-choline for 1 h, ... were then washed for 5-min periods and ... metabolites were separated by ... with ... and by paper ... Release of acetylcholine ..., choline (O O) and the unprecipitated fraction (—). The graphs show ... S.E. of the mean from 5 different

the expts. the release of radioactivity during a collection period was expressed in per cent ... total amount of radioactivity calculated to be present in the tissue at the beginning of ... expt. This was considered preferable to expressing the release in pmol/mg wet tissue ... for three reasons. Firstly the specific activity of the metabolites is not known since ... might be diminished by dilution of endogenous substances. Thus it was not possible to ... the values with great accuracy by the latter method. Secondly the variation between ... experiments was found to be smaller with the use of the percentual value, possibly ... to a normalization of the values by this method with respect to the actual amount of ... activity taken up by the tissue. Thirdly the percentual value has the advantage that it ... directly to the amount of radioactivity that can be released from the preparation.

During the first washing period radioactivity was rapidly lost from the tissue. This was ... due to a rapid washout of ^{3}H-choline, but also to a lesser extent to the release of the ... precipitated fraction. After the initial period the losses of all metabolites were less drastic ... 2).

Regression analysis. Analysis of the washout curves of ^{3}H-choline and the unprecipitated ... fraction by the graphical curve peeling technique (Casteel and Droogmans 1975) indicated ... these curves consisted of two exponential decays. Analysis of the release of ^{3}H-acetyl... choline by the same method was not as straightforward, this release showing greater fluctua... than that of the other metabolites. It could only be stated, therefore, that the release ... ^{3}H-acetylcholine was probably best compatible with a biexponential model.

On the assumption that the release of all metabolites followed biexponential courses, the ... were fitted into equation (2) by non-linear least square regression (see Methods, p. 305). ... results of the determined constants are shown in Table I A.

The analysis indicated that the studied metabolites were released from 2 different compartments. (The two compartments of a particular metabolite were not, of course, necessarily ... same as those of another metabolite.) It is not possible to arrive at a single physical inter... pretation of the organization of the compartments from the experimental data. Several different models would give rise to biexponential time courses in the release of radioactivity ... speculations may be made, however, about the physical nature of the different compart

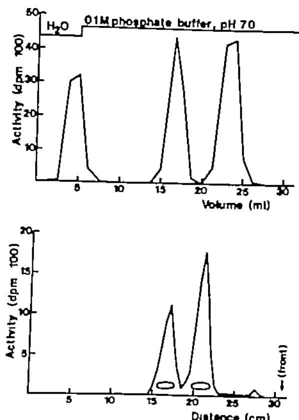


Fig. 1 A. Column chromatography of 1 ml of washing solution on Amberlite CG 50. The unbound fraction is eluted by water. The acetylcholine and choline peaks are eluted by 0.1 M phosphate buffer pH 7.0. B. Paper chromatography of total quaternary ... acetone/water/10 N HCl (100:20 ...). The chromatogram was cut into 0.5 cm ... counted in Instagel after the radioactivity had been eluted off the paper by 3 ml ethanol/water (1:1). The peak of H-acetylcholine appears in front of the H-choline peak. The spots of carrier acetylcholine and choline are indicated below the peaks.

Separation of the ammonium-reineckate precipitated bath solution by paper chromatography yielded 2 peaks with acetone/water/10 N HCl with R_F values of 0.71 and 0.... The R_F of native acetylcholine was found to be 0.69 and of choline 0.57 (Fig. 1 B). In butanol/water/ethanol/acetic acid, again two peaks appeared with R_F values of 0.46 and 0.3... The R_F of native acetylcholine was 0.46 and of choline 0.36. More than 95 of the radioactivity on the chromatograms was estimated to consist of choline and acetylcholine. Since the acetone/water/HCl system showed the most favourable R_F values, this system was used in all paper chromatographic separations in expts. reported below

Kinetic of the release of tritiated metabolites under unstimulated conditions

Five minute fractions were collected for 1.5 h after the incubation period and the radioactivity in the Krebs solution was separated into 3 components. The *TQA* content was determined by the radioactivity in the reinecke precipitate of the wash solution. The acetylcholine and H-choline in this fraction were separated by paper chromatography. The radioactivity of the metabolites which were left unprecipitated by ammonium reineckate was obtained by calculating the difference between the radioactivity of the washing solution and the *TQA* activity. In the following this fraction will be called the unprecipitated fraction (*UPF*).

After the incubation, 413 ± 46 pmol (7.88 ± 0.4? pmol/mg wet tissue) (S.E. of mean, n = 5) of the radioactivity was retained in the tissue. This amount corresponded to an uptake of 20.9 ± 2.3 % (S.E. of mean) of the total amount of radioactivity added to the incubation medium at the beginning of the expt.

[illegible] II Estimates of extra- and intracellular compartments of radioactivity in the guinea pig ileum after incubation with ^{3}H-choline. The calculations were based on the data from Table I A under the assumption that the metabolites were released in cat... systems.

	Extracellular ( )	Intracellular ( )
[illegible]	3.74 ± 0.57	5.03 ± 0.22
[illegible]choline	1.72 ± 0.38	10.8 ± 5.0
	1.07 ± 0.09	5.43 ± 0.93
[illegible] (by difference)	—	72.2 ± 5.6

[illegible]e at different times int eq. (1) (see Methods, p. 304) on the assumption that the losses [illegible] the metabolites could be approximated to a biexponential decay. The results are shown [illegible]able I B, and Fig. 3. The non-releasable stores arrived at by the regression analysis were [illegible] 1.3% (S.E. of mean), which was close to the value calculated above.

[illegible]*orrelation between* ^{3}*H-acetylcholine release and isometric tension.* Although eserine [illegible] 10^{-4} M), which was present throughout the expt., induced a strong contraction of the [illegible]paration, spontaneous variations of the isometric tension were observed. Since the release [illegible] ^{3}H-acetylcholine also showed spontaneous fluctuations, it was of interest to test the cor[illegible]tion between the two variables. As a suitable measure of the acetylcholine release, the [illegible]iduals from the regressions were used. (A residual is the distance from a data point to the [illegible]ression curve, see Draper and Smith 1966.) The deviation from the mean tension cal[illegible]ated for the whole experiment was used as measure of the isometric tension.

[illegible] strong positive correlation ($= 0.64$ $p < 0.001$) between the two variables, from all the [illegible]ta points (n 90) in five different expts., was found (Fig. 4).

[illegible]*lease of metabolites during electrical field stimulation*

[illegible]e studies of the kinetics of ^{3}H-choline release from the preincubated preparation indicated [illegible] essentially all extracellular metabolites had disappeared after a washing period of 30 [illegible]in. For studying the effect of electrical stimulation, the preparation was therefore washed [illegible] this length of time before any fractions were collected. After this 30 min 380 ± 37 pmol [illegible] 0.98 pmol/mg wet tissue) (S.E. of mean n 5) of the tracer was retained in the tissue. [illegible]his amount corresponded to an uptake of 19.2 ± 1.8% (S.E. of mean) of the radioactivity [illegible]esent at the start of the incubation.

[illegible] *Separation of metabolites by paper chromatography.* Electrical field stimulation by 150 [illegible]lses with a duration of 1 ms at 0.5 Hz given for 5 successive 5 min periods induced a large [illegible] consistent increase in the release of ^{3}H-acetylcholine (75.0 ± 15% (S.E. *of mean*, $n = 25$) [illegible] the total increase of radioactivity induced by the electrical stimulation) (Fig. 5). The [illegible]lectrical stimulation also resulted in some increase in *UPF* amounting to 18.0 ± 1.2% [illegible]S.E. of mean, n 25) of the total increase. Only a minor increase in the release of ^{3}H-[illegible]holine (7.0 ± 1.3% (S.E. of mean, $n = 25$) of the total increase) occurred on electrical [illegible]timulation.

[illegible] *Separation of metabolites by ion exchange chromatography.* Separation of the metabolites [illegible] by ion exchange chromatography gave similar results as paper chromatography. Electrical

TABLE I A. Estimated constants of equation (2) derived from nonlinear least square regression of ... expts. of choline acetylcholine and UPF B. Estimates of constants of equation (1) ... activity calculated to be present in the tissue during the washout expt. (The expts. were ... formed under unstimulated conditions.)

Metabolite	A (%)	α (min)	A_1 (%)	α (min^{-1})	I(
A					
Choline	3 30 ± 0 35	0 378 ± 0 009	5 47 ± 0.23	0.0150 ± 0 001	–
Acetylcholine	0.574 ± 0 067	0 120 ± 0.018	11.9 ± 4.9	6 40 ± .18 10^{-3}	–
UPF	0.805 ± 0.078	0.388 ± 0 030	5 70 ± 0.93	9 54 ± 1.52 10^{-3}	–
B					
Total	4 76 ± 0.37	0.303 ± 0.009	17 1 ± 0.98	0 0102 ± 0 0010	31

ments. It is probable that the initial rapid release was due to a washout of the extrac space. The slower components might be derived from intracellular compartments. U certain assumptions concerning the models, it might be possible to arrive at a single sol for the magnitude of the different compartments and the velocity constants of exchange metabolites between the compartments and between the compartments and the wa solution. Owing to the uncertainty of what assumptions would be most compatible reality however it is not possible to argue much on this point. The amount of radioac present in the compartments at $t = 0$ will, however be discussed If one compartmen located extracellularly while the other was confined to the interior of the cells, the r of the metabolite from the intracellular compartment would probably have occurred extracellular space. The extracellular space would then release its activity to the surrou Krebs solution. In this case the release would occur in a series, and a catenary system then be present The solution of a two-compartment catenary system was quoted by C and Droogmans (1975) from Huxley (1960) The amount of radioactivity present in d ferent compartments, as calculated by these formulas, is shown in Table II In all ca radioactivity present in the intracellular compartments was greater than that in the cellular one. The largest pool was found in the intracellular acetylcholine depot. Of the cellular radioactivity the largest amount was found in the choline fraction. No qualit different results from these would be derived if it was assumed that a parallel (mamm system was present instead of a catenary one In the mammillary system the amount p in a compartment at $t = 0$ would be given by the A_i values in Table I A.

Without further arguing for or against any particular physical model of the ob relations it is possible to derive more information concerning the compartmentalizat radioactivity in the tissue. Irrespectively of which model is chosen, the sum of all A_i from the regressions can be shown to represent the total amount of radioactivity p in all releasable compartments at $t = 0$ (see Methods, p. 305 eq 3) This amount w culated to be 27 7 ± 5 6% (S E. of mean). Thus 72.2% of the initial 100 of radioa in the tissue was lacking, which meant that a rather large amount of radioactivity und unstimulated condition prevailing, consisted of non-releasable stores. This was furthe ported by fitting the data from the (calculated) amount of total ity present

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Metabolite	A_1 (%)	α_1 (min⁻)	A_2 (%)	α (min⁻¹)	[illegible]
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B					
Total	4 76±0.37	0 303±0 009	17 1±0 98	0.0102±0 0010	[illegible]

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Without further arguing for or against any particular physical model of the observed relations it is possible to derive more information concerning the compartmentalization of radioactivity in the tissue. Irrespectively of which model is chosen, the sum of all A_i values from the regressions can be shown to represent the total amount of radioactivity present in all releasable compartments at $t = 0$ (see Methods, p. 305, eq. 3). This amount was calculated to be 27 7±5 6% (S.E. of mean). Thus 72.2% of the initial 100% of radioactivity in the tissue was lacking, which meant that a rather large amount of radioactivity under the unstimulated condition prevailing, consisted of non releasable stores. This was further supported by fitting the data from the (calculated) amount of total radioactivity present in

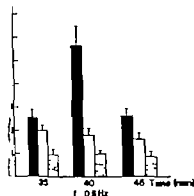


Fig. 6

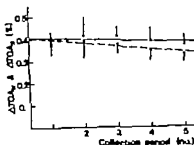


Fig. 7

Fig. 6. Washout of radioactivity from the preincubated guinea pig ileum. The metabolites were separated by column chromatography on Amberlite CG 50 II. Acetylcholine is indicated by filled, choline by unfilled and the unbound fraction by shaded columns. Electrical field stimulation at 0.5 Hz was performed during second collection period ($n = 5$).

Fig. 7. Release of acetylcholine due to stimulation calculated as ΔTQA_x (—) and calculated as ΔTQA_c ($n = 5$).

during the 5 stimulation periods in the expt. described above, is shown. ΔTQA calculated in per cent of the total radioactivity present in the tissue at the beginning of the expt. showed a slight decrease during the course of the stimulation periods. This is of course to be expected, but if, for instance, the effects of drugs were to be studied, it would be desirable that ΔTQA remained constant. By instead, calculating the release as the ΔTQA in per cent of the radioactivity present in the tissue at the beginning of the stimulation period, the release remained constant (Fig. 7).

Correlation between number of pulses delivered and ΔTQA. To investigate whether there was any correlation between the number of pulses delivered to the preparation and the release of TQA, the preparation was stimulated by 30, 90, 300, 900 and 3 000 pulses with a duration of 1 ms for 5 min. Even the smallest number of pulses induced a clear increase of the TQA

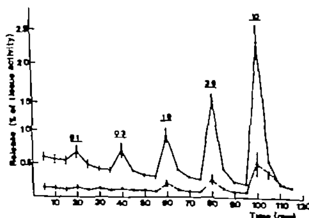


Fig. 8. Washout of radioactivity from the preincubated preparation. The metabolites are separated by precipitation with reineckate. TQA (●—●) and the unprecipitated fraction (○—○). Electrical field stimulation at 0.1, 0.3, 1, 3 and 10 Hz for 5 min is indicated by horizontal bars ($n = 5$)

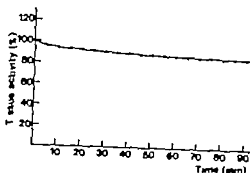


Fig. 3

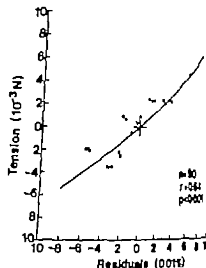


Fig. 4

Fig. 3 Loss of radioactivity from guinea pig ileum preincubated with H-choline. The preparation was washed for 5-min periods and the radioactivity present in the tissue was calculated from the amount released to the washing solution. The continuous line represents the regression of equation (I) to the data.

Fig. 4 Correlation between spontaneous release of H acetylcholine and the isometric tension of the preparation. The acetylcholine release was expressed as the deviation from the regression of the washout curves (e g as the residuals from the regressions). The tension was expressed as the deviation from the mean tension in each expt.

field stimulation by 150 pulses with a duration of 1 ms at 0 5 Hz induced an increase in the release of acetylcholine similar to that in the previous expt. The electrical field stimulation did not affect the release of H-choline while the unbound fraction showed a slight tendency of increase (Fig. 6)

Measurement of stimulation evoked acetylcholine release as ΔTQA The expts. described above indicated that measurement of *TQA* was of equal value to measurement of acetylcholine itself in estimating the stimulation evoked release of acetylcholine, the reason being that the release of choline was almost unaffected by the electrical stimulation. In Fig 7 the magnitude of the increase in *TQA* (Δ*TQA*) due to stimulation by 150 pulses at 0.5 Hz of

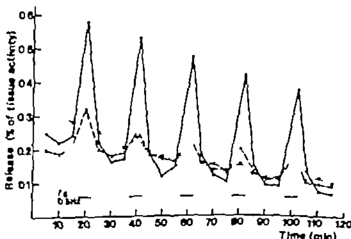


Fig 5 Washout of radioactivity from the preincubated guinea pig ileum. The metabolites were separated by precipitation with reineckate and by paper chromatography Acetylcholine (●—●) choline (O O) and the unprecipitated fraction ( ) The electrical field stimulation at 0.5 Hz for 5 min (—) (n = 5).

ar metabolism

raction induced by low-frequency field stimulation in the longitudinal guinea pig ileal is known to be mediated by stimulation of cholinergic neurons. It has been observed these contractions diminish in amplitude after prolonged stimulation periods. If choline (1 mM) is added to the incubation medium, the contractions can be restored to their ial levels (Kosterlitz, Lydon and Watt 1970). These results might indicate that extra-lar choline is an important precursor for the synthesis of acetylcholine by the nerves and the metabolism of the preparation itself is not sufficient to supply the nerves with ne.

e present study demonstrates that the guinea pig ileum takes up tritium when it is in-ted ith ³H-choline. If the isotope is distributed equally in the tissue and the incubation um, the expected uptake may be estimated to be roughly 1%. The alue obtained rimentally however was approximately 20%, indicating that the uptake is an active ess.

absolute figures, about 7.9 pmol of radioactivity per mg wet weight was taken up and ined by the tissue after 1 h of incubation with 1 μM ³H-choline. Szerb (1975) arrived at a lar result. He calculated that 8.8 pmol radioactivity per mg was taken up by the longi-nal guinea pig ileum after perfusion for 54 min with ³H-choline at the same concentra- as was used in the present expts. The slightly lower uptake in the present study might due to the fact that the preparation was incubated with a smaller amount of H-choline n in the expts. of Szerb. From his results, the contents of endogenous acetylcholine and line of the preparation could be calculated to be about 100 and 29 pmol/mg, respectively eserine was present. A similar acetylcholine content (120 pmol/mg), could be cal-ated from the results of Paton and Zar (1968). The difference between the amount of ogenous metabolites and the amount of radioactivity retained in the tissue indicates that label was probably diluted to a large extent by endogenous substances. This also in-ated that there is little justification for expressing the release of radioactive metabolites absolute figures as long as their specific activities are not known. The problem could haps be overcome by incubating the preparation at higher concentrations of H-choline, reby supposing that the dilution would become negligible. However since the sole pur-e of the present study was to find a method for monitoring changes of acetylcholine ease, no such attempts were made.

nthesis of H-acetylcholine

e present study confirms the results of Szerb (1976) that the preparation preincubated with choline released ³H-acetylcholine both during rest and on electrical stimulation when serine as present. I this work the identity of the ³H-acetylcholine was established with no doubt, since the metabolite showed identical behaviour to that of native acetylcholine three chromatographic systems. Thus, it was demonstrated that choline could serve as a precursor in the synthesis of acetylcholine in the guinea pig ileum.

Evidences have been presented that two choline transport systems are present in the guinea pig ileum. On incubation with ³H-choline high affinity site with a K_m of 2–4 μM d a low affinity site with K_m of 90–100 μM was observed. Furthermore, the high affinity

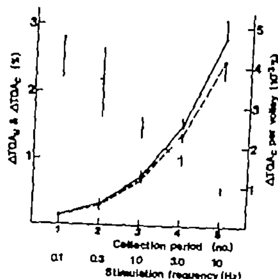


Fig. 9 Release of acetylcholine due to electrical stimulation, calculated as ΔTQA_c (—), ΔTQA_c (- -) and as ΔTQA_c released per volley ( ) during stimulation at 0.1, 0.3, 1, 3 and 10 Hz for 3 min ...

release (Fig. 8). On increasing the number of shocks delivered, a close correlation to the increase in the release of *TQA* was observed. The data were also corrected for loss of tritium by the method described above. The results are shown in Fig. 9 together with the uncorrected data for comparison.

It has been shown by Paton and Zar (1968) that by increasing the stimulation frequency in the guinea pig ileum an increase in the total release of acetylcholine is obtained. If the release of acetylcholine is calculated, instead, as the output per delivered pulse, the output is in contrast smaller at high stimulation rates than at low. A similar observation was made in the present expt., *i.e.* the release of ΔTQA per volley diminished at higher stimulation rates (Fig. 9).

Discussion

This investigation was started with the intention of finding a simple and fast method for studying acetylcholine release from the mammalian intestine. For reasons pointed out in the introduction, bioassay was excluded. As H-choline of high specific activity could be obtained, another approach became possible. It seemed that if the acetylcholine stores of the nerves in the preparation could be labelled by H-acetylcholine detection of the neurotransmitter release might be fairly simple.

Isolated longitudinal guinea pig ileum was used for the study. This organ was found to be well suited for investigating cholinergic mechanisms by the radiochemical method. The preparation consists of the main parts of the Auerbach's plexus adherent to the longitudinal smooth muscle. A few circular muscle fibres may also sometimes be observed (Paton and Zar 1968, Wikberg 1976). Thus, the only part of the intestinal nerve plexus that is studied in this preparation is the nervous network between the longitudinal and circular muscle. Other tissue layers such as the intestinal mucosa are not present, otherwise these would probably have introduced difficulties in interpretation of the results. The presence of smooth muscle in addition to the nerves, must however be taken into account.

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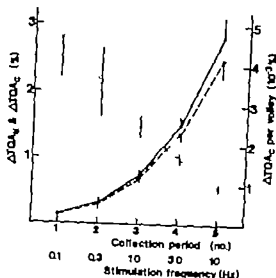


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...s strong as for the other metabolites. It is probable that the metabolites were released ...g the incubation, thus accumulating extracellularly. This accumulation of extracellular ...bolites might then be the basis of the rapid phase of the efflux. The amounts of radio...ity in the acetylcholine and in the unprecipitated fraction, present in the extracellular ...artment, seemed to be small in comparison with the other pools of radioactivity ...s suggested that the slow components of the release are derived from intracellular sites. ...elease of acetylcholine from this compartment is discussed further below. The mechan... of the release of the metabolites in the unprecipitated fraction is unknown. The release ... perhaps be derived from metabolism of more tightly bound stores of radioactivity in ...ssue.

...tween 72 and 78% of the radioactivity in the unstimulated preparation, consisted of ...eleasable stores. This finding should not necessarily mean that the stores were strictly ...releasable; thus, this compartment might represent a release of radioactivity with a very ... half time. During a short period (1.5 h) such a pool would appear as non-releasable. ...how these stores were observed as a tightly bound pool of radioactivity in the tissue. ...ther or not this pool can be released upon prolonged electrical stimulation remains to ...tablished. Szerb (1976) suggested that two populations of cholinergic neurons were ...ent in guinea pig ileum. One of these populations released its acetylcholine content ...ng spontaneous conditions or during low frequency stimulation. The other population ... only activated at high rates of stimulation. The latter population of neurons might at ... partly be responsible for the non-releasable store observed in the present work.

...is also possible that the non-releasable stores consisted of choline incorporated into ...spholipids, suggested by Collier and Lang (1969) to be synthesized from H-choline in ...pathetic ganglions. However, the trichloroacetic acid insoluble fraction which ap...red after 54 min of incubation with ³H-choline at 1 µM in the study of Szerb (1975), ...ately seems to be t... small to completely account for the non-releasable pool in the ...sent experiment.

...ylcholine release

...s well known that intestinal preparations have spontaneous contractile activity of varying ...plitude and frequency. In the guinea pig ileum these contractions, when they occur, are ...nhanced by eserine (3 · 10⁻⁶ M) and blocked by atropine (3.5 · 10⁻⁷ M) (W...kberg, un...blished observations), which indicated that the parasympathetic system plays a role in the ...ontaneous intestinal motility of this organ. Under unstimulated conditions the release of ...acetylcholine showed spontaneous fluctuations. The variations were much greater than ... variation in the release of ³H-choline or of the unprecipitated fraction. It is not improb...le that the spontaneous fluctuations of the acetylcholine release could be due to spon...neous variations in the activity of the cholinergic neurons of the preparation. A good cor...lation between the release of acetylcholine and the mechanical tension was also observed. ...s found possible to arrive at this correlation even though the strong contraction in...ced by eserine probably obscured some of the variations of the intestinal tone. These ob...rvations combined make ... fairly coherent picture and indicate the consistency of the radio...emical method for the detection of ³H-acetylcholine.

site disappeared when the preparation was denervated while the low affinity site was present after this procedure (Pert and Snyder 1974) In a study of the present type it would [illegible] desirable for the acetylcholine stores of the nerves to be selectively labelled, as this would reduce the disturbance from other metabolites released from the preparation. The data [illegible] Pert and Snyder indicate that a low concentration (0 4–1 μM) of ^{3}H-choline should be used when incubating the preparation, in order to take advantage of the high affinity uptake system of the neurons Both their results and the results of Szerb (1975) indicate that this will lead to a larger synthesis of ^{3}H-acetylcholine relative to other metabolites, than when high concentrations (50–100 μM) of ^{3}H-choline are used

Other metabolites Besides choline and acetylcholine other metabolites were also released from the preparation It is probable that these metabolites, which were named the [illegible] precipitated and the unbound fraction represented the same substances, since the [illegible] fraction disappeared after precipitation by reineckate

At the present moment the identity of the unbound and the unprecipitated fractions is not known Their nature may be speculated upon, however They are probably not strongly positively charged, since they were not bound by the Amberlite column. It is also probable that they are not quaternary cations, since these might be expected to be precipitated by reineckate ions Possible candidates for the metabolites are therefore less strongly charged molecules such as N N dimethylethanol-amine or zwitterions such as phosphorylcholine and betaine. Szerb (1976) suggested that the fraction remaining after precipitation with reineckate, which was released from guinea pig ileum, represented ^{3}H-phosphorylcholine since this metabolite was identified in extracts from preparations preincubated with ^{3}H-choline The definite settlement of the nature of UPF should, however await its [illegible] chemical identification in perfusate

Compartmentilization of tritiated metabolites From the kinetic analysis of the released ^{3}H-choline it was apparent that the metabolite was released with a biexponential time course the half times being 1 8 and 46 min respectively It is suggested that the metabolite was released from two different compartments, an extracellular and an intracellular It is [illegible] understandable why ^{3}H-choline should be released in large quantities from the extracellular space This extracellular choline could represent the choline of the incubation medium which was not taken up by the tissue and not washed away by the first brief rinse of the preparation The source of the slow component of choline release is at present not clear This choline could be derived from the nerves as well as from the smooth muscle of the preparation [illegible] could represent a breakdown of acetylcholine by esterase not blocked by eserine. The choline could also be derived from an intracellular pool of free choline It was suggested that such choline might have been present in the cat superior cervical ganglion after perfusion with tritiated choline (Collier and MacIntosh 1969)

Efflux studies on the isolated rat diaphragm indicated similar conditions to those in the guinea pig ileum. The rat diapraghm released ^{14}C-choline from two different compartments with half times of 2.5 and 36 min, when the preparation had been preincubated with labelled choline (Potter 1970)

There was little doubt that the unprecipitated fraction was released from two different compartments. This could also be true for acetylcholine although the evidence for this [illegible]

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In this work the spontaneous release of ^{3}H-acetylcholine ranged between 0.097-0.03[illegible] of the total stores of radioactivity present at the beginning of the experiment. From the [illegible] of Paton and Zar (1968) the release of acetylcholine in the longitudinal ileum [illegible] calculated to be 0.34%/min of the acetylcholine content in the preparation. The acetylcholine release in the present study and that in the study by Paton and Zar are not [illegible] comparable, as they were calculated by different methods. However the results do not seem to be grossly discrepant, since the release calculated in this work may be expected to be lower than the value derived from Paton and Zar (1968). The previous value refers not only to the acetylcholine content of the tissue but also to other metabolites present in the tissue.

Stimulation-evoked release. Electrical field stimulation increased the release of ^{3}H-acetylcholine from the preparation. The ^{3}H-choline release was increased only slightly or not at all by the electrical stimulation and the release of the unknown metabolites may [illegible] to some extent.

Since the electrical stimulation almost exclusively affected the release of the acetylcholine and not the release of the choline, and since the *TQA* was found to contain only these [illegible] metabolites, it is suggested that measurement of *TQA* is of equal value to measurement of ^{3}H-acetylcholine in the study of stimulation-evoked release of the neurotransmitter. Measurement of *TQA* is preferable as the chromatographic procedures are rather time consuming. This approach will therefore considerably reduce the work involved in the method. It must, however, be emphasized that under unstimulated conditions, determination of *TQA* [illegible] is probably of limited value, as the partition of acetylcholine and choline cannot then be predicted.

One of the most important findings in the present work was that the stores of radioactivity in the preparation, were sufficiently great to allow repeated stimulation of the preparation and still obtain a large release of ^{3}H-acetylcholine. The release of ΔTQA decreased naturally slightly on repeated stimulation at constant frequency. By calculating the release of ^{3}H-acetylcholine as ΔTQA a correction was obtained, so that the release remained almost constant. This finding could be of importance in studies of the influence of drugs, for example, on the stimulation-evoked release of acetylcholine.

The release of total quaternary amines on electrical stimulation at different rates was similar to the release of acetylcholine under similar conditions reported by Paton and Zar (1968). The increase of ΔTQA was greater at high rates of stimulation, but the increase per volley diminished. The similarity between the results of this study and those of Paton and Zar strengthens the validity of the present method.

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apacity of mouse skeletal muscle to oxidize radioactively labelled fatty acid and the tionships of the fatty acid oxidation capacity to the other metabolic pathways. The y was first conducted with "mixed" skeletal muscle and then extended to comprise the ts of training in predominantly red and predominantly white muscle tissue.

Material and Methods

and training

tudy (EXP I) as first conducted with young (2 months old at the beginning of training) male ice. The training animals (N = 11) were running 30 min/day 4 days eek during 5 months on ven treadmill at speed of 20 m/min. Control animals (N = 9) lived in normal cage conditions. second expt. (EXP II) male F_1-hybrids (B6D2F1/BOM, Gl Bomholtgård Ltd, Denmark) of the months at the beginning of training were used. The training animals (N = 35) ere running 1 7 days week, 3 times per day 30 min at speed of 20 5 m/min, 45 min at speed of 17.6 m/min min at speed of 16 6 m/min. Control animals (N = 27) lived in normal cage conditions.
third expt. (EXP III) male NMRI mice of the age of 2 months were used. The training group ran 5 days week 60 min per day for 4 months at speed of 17.6 m/min. Control animals lived in normal cage conditions.
nimals were given commercial pellet food (Hankkija, Finland) and ater *ad libitum*. Animals ere in 46 23 13 cm cages (8–10 mice in each cage) in room where temperature and humidity onstant. The day was artificially divided into 12 h of ligh and 12 h of darkness changing at 6 00 e training of animals was performed before noon on fixed time in each experiment series.

amples

were trained up until the day before sacrifice. During each expt. day 2 or 3 control and trained were studied. The animals were sacrificed by dislocation of the neck and the muscle sample under quickly excised and weighted. In EXP I and II the whole *m quadriceps femoris* (MQF) as used EXP III the right MQF as divided into grossly red and grossly hite parts while the left MQF essed as in EXP I and II. The mainly red part consisted of the proximal heads of *m vastus* and *m vastus medius* and the red fibers of *m vastus intermedius* Thus grossly red sample as not tly red" because the superficial parts of *m vastus medius* and *m. vastus lateralis* contain also fibers. The grossly red part of control mice weighted 77 6 ± 3 7 (S D) mg and that of trained mice 9 mg. The predominantly hite part consisted of the distal heads of *m vastus lateralis* and *m. femoris*. In controls this sample weighted 66 9 ± 6 5 mg and in trained animals 66 2 ± 4 1 mg.
sample was cut with scissors into smaller pieces and homogenized in Potter Elvehjem homogen th teflon pestle in ice-cold buffer (175 mM KCl, 0 1 mM EDTA, pH 7 4) The homogenization in one minute ith 4–6 complete passes of the pestle. The preparate as made 2 and an aliquot 3 0 ml) was removed for the measurement of fatty acid oxidation capacity (FAO).

ement of fatty acid oxidation capacity

ghtly modified reaction mixture for FAO measurement (Mole *et al* 1970) contained 5 mM $MgCl_2$, M KCl, 40 mM potassium phosphate buffer (pH 7 4), 2 mM EDTA, 25 μM coenzyme A, 1 mM ne, 2 mM ADP and 70 μM palmitate as NH_4-salt The proportion of carboxyl-labelled (New d Nuclear Corp) palmitate as 5 7 The radioactivity of the substrate was in EXP I 300 000 dpm EXP II and III 700 000 dpm per 0 11 μmol palmitate.
reaction was carried out in liquid scintillation counter bottles (25 0 ml), which were in water bath (37°C) under light shaking The reaction as started by addition of 1 0 ml homogenate (20 mg t) and the vessel was closed ith rubber stopper After 30 min the reaction was stopped by in 0 5 ml 60 citric acid into the reaction bottle The delivered carbon dioxide was washed by oxygen the "trap" bottle containing 0 5 ml ethanolamine-methylcellosolve (1 1) absorbed on piece of paper At the beginning of 1 1.5 h ashing the oxygen flow rate as 10–15 ml/min and the flow was y accelerated during the ashing The trap as very effective since over 93 % of $^{14}CO_2$ was detected trap vessel already after 30 min flow The filter paper was folded and 10 ml of scintillation liquid g PPO, 3 mg POPOP 2 ml methylcellosolve, 8 ml toluene) as added and the radioactivity as

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Effect of Endurance Training on the Capacity of Red and Wh[illegible] Skeletal Muscle of Mouse to Oxidize Carboxyl ¹⁴C-labelled Pal[illegible]

By

ANTERO SALMINEN VEIKKO VIHKO and LARS PILSTRÖM

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Abstract

SALMINEN A V VIHKO and L. PILSTRÖM. *Effect of endurance training on the cap[illegible] red and white skeletal muscle of mouse to oxidize carboxyl- C-labelled palmita[illegible]* physiol. scand 1977 *101* 318-328.

Three groups of mice were trained for 1 4 and 5 months according to different running pro[illegible] a motor driven treadmill and the fatty acid oxidation capacity (FAO) and the acti[illegible] ities of some e[illegible] energy metabolism (cytochrome c oxidase, malate dehydrogenase, triosephosphate dehydroge[illegible] lactate dehydrogenase) were determined from *m quadriceps femoris* (MQF). Endurance training [illegible] the FAO (5-month tra ning, 4 days/week 30 min/day [illegible]2% ($p<0.05$) 1-month training, 7 d[illegible] 150 min/day 37 ($p<0.001$); 4-month training, 5 days/week, 60 min/day 4 [illegible] ($p<0.05$)). The ac[illegible] cytochrome c oxidase and malat dehydrogenase increased approx. 30% ($p<0.001$) whereas tr[illegible] phate dehydrogenase and lactat dehydrogenase activities were not prominently influenced by [illegible] The predominantly red part of MQF of untrained animals oxidized palmitate four times faster [illegible] predominantly white part. The act vities f cytochrome c oxidase and malat dehydrogenase [illegible] times higher showing pronounced FAO n the red part. Endurance training increased the activities of oxidative enzymes in the red and whit parts nd i the whole muscle relative[illegible] resulting in similar d fferences between the muscle types after training. The absolute increase in th[illegible] the red muscle was, however ma yfold when compared n chemical units to the white musc[illegible]

Key words: Red/whit skeletal muscle palmit t o idation endurance training, enzyme activiti[illegible]

Endurance training adapts the metabolic capacities of exercised skeletal muscles. T[illegible] important adaptation is the increase in the oxidative capacity of muscle This is [illegible] as increased activities of the enzymes of citric acid cycle (*e.g.* Holloszy 1967), β-o[illegible] (Mole *et al* 1971 Baldwin *et al* 1972) and respiratory chain (*e g* Holloszy 1967 [illegible] *et al* 1972).

Fatty acid oxidation provides the major part of energy utilized during aerobic en[illegible] performances (Keul *et al* 1972). In the mammalian skeletal muscle red fibers a[illegible] specialized for oxidative energy production and they are recruited during prolong[illegible] [illegible] In the following study we investigated the effects of endurance type of trai[illegible]

...ate dehydrogenase activity (LDH) (E.C. 1.1.1.27) was used as measure for the anaerobic capacity of muscle. The assay was performed according to Kornberg (1955) by following the oxidation of NADH (0.2 mM) at 340 nm in the presence of sodium pyruvate (5 mM) in 50 mM phosphate buffer (pH 7.5) and 25 µl homogenate dilution (1:10) in final volume of 0.925 ml at 22°C.

Malate dehydrogenase activity (MDH) (E.C. 1.1.1.37) was, like CytOx, used as marker for oxidative capacity. The reaction mixture (Ochoa 1955) contained 25 mM Tris-HCl pH 7.4, 0.3 mM oxaloacetate, ... mM NADH and 25 µl homogenate dilution (1:10) in final volume of 0.925 ml at 22°C.

...phosphate dehydrogenase activity (TPDH) (E.C. 1.2.1.12) was used as measure for the ...lytic capacity of the muscle. The reaction mixture (Bass et al. 1969) contained 4 mM EDTA, 40 mM ..., 26.5 mM $MgSO_4$, 1.1 mM ATP, 5.1 mM D-3-phosphoglycerate, 1.8 mM glutathione, 0.16 mM NADH, ... µl phosphoglycerate kinase (20 µg, Biochimica Boehringer) and 25 µl homogenate dilution ... in final volume of 0.685 ml at 22°C.

Enzymatic activities are referred per muscle wet weight and per protein basis. Protein concentration was estimated according to Lowry et al. (1951). In EXP II the homogenates were hydrolyzed before ... assay in 1 N NaOH at 40°C for 1 h. Bovine serum albumine (Sigma) was used as protein standard.

Statistical methods

Standard procedures were used to calculate means and standard deviations (SD). The significance of differences between means were calculated by Student's t-test. In addition correlations between different variables were determined by the least square method.

Results

Effects of endurance training on FAO in MQF

Endurance training increased the capacity of MQF of mouse to oxidize palmitate in vitro. In the pilot study (EXP I) the FAO of trained mice, expressed on protein basis, was higher ($p < 0.05$) than in controls. Per wet weight the difference was 23.5%, but not statistically significant (Table I).

The more intensive training of F_1-hybrid mice in EXP II caused markedly greater changes (Table II). The FAO of controls was 41.9 ± 6.6 and that of trained mice 57.2 ± 11.1 ...min/mg muscle wet weight. This difference was 36.5% ($p < 0.001$). When calculated on protein basis the increase was even higher (40%, $p < 0.001$). CytOx activity increased ...% (p 0.001), i.e. less than FAO capacity. LDH activity decreased but the change was rather small and significant only in case of activity per muscle wet weight (Table II).

In EXP III the FAO in MQF of controls was 41.7 ± 8.4 and that of trained 51.8 ± 14.9 ...min/mg wet weight. The increase was 24.2% (p 0.05). CytOx ($p < 0.05$) and MDH

Table I. EXP I. The weights of control and trained animals, the weights and protein contents of m. quadriceps femoris (MQF) and the fatty acid oxidation rates (FAO) (mean ± S.D.) together with the per cent differences between control and trained animals (%) and the significance (p) of the difference.

Variable	Controls (n = 9)	Trained (n = 11)	Δ%	p
Weight of animals (g)	36.5 ± 2.7	34.9 ± 2.6	− 4.5	
Weight of MQF (mg)	271 ± 23	260 ± 24	− 4.2	
Protein content[a] of MQF	163 ± 28	166 ± 20	+ 1.8	
FAO[b] 10^3 muscle wet weight	31.5 ± 8.4	38.8 ± 7.8	+ 23.5	
FAO[b] 10^3 muscle protein	192 ± 43	235 ± 43	+ 22.4	< .05

[a] Protein content: µg protein/mg muscle wet weight.
[b] FAO: dpm/min/mg muscle wet weight or muscle protein.

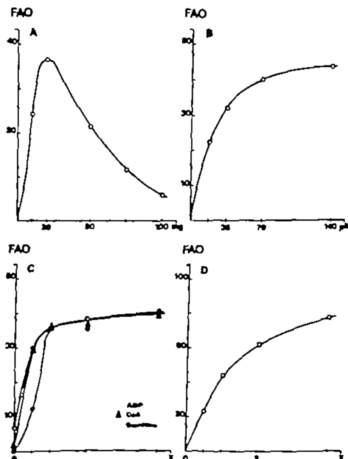


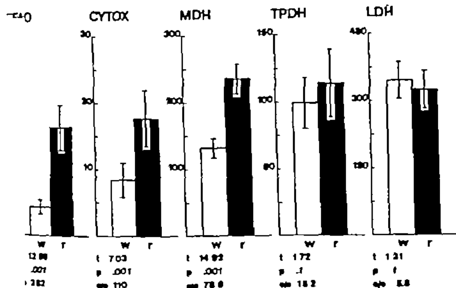
Fig. 1 Some testing results of FAO measurement. Values for FAO are expressed as dpm/min/mg muscle. The optimum amount of muscle. The effect of NH_4-palmitate concentration on FAO in the routine method. C. The effects of concentrations of cofactors (ADP CoA, carnitine) on FAO. In the test series the concentration of one cofactor at a time has been doubled etc. while the other has been the same as in the routine method. D The effect of manyfold concentrations of cofactors on FAO.

measured with Wallac scintillation counter Double assays per each muscle sample were made. The method error was approximately 5%.

In Fig. 1 some test results of the FAO measurement method are given. The highest FAO was achieved with 20 mg of muscle (Fig. 1 A) when the protein content in the reaction mixture was approximately 2.5 mg/ml. Optimal reaction time was 30 min, longer incubation slightly decreased the FAO. Almost maximal oxidation rate was achieved by substrate concentration 70 μM palmitate (Fig. 1 B) which was used in the routine assays. Michaelis-Menten constant (Km) was then approx. 15 μM. The effects of ADP CoA and carnitine on the oxidation rate were also checked (Fig. 1 C, D). ADP was the most important activator. The cofactor concentrations used in the routine method gave almost maximal oxidation rate (Fig. 1 C), although manyfold addition of ADP CoA and carnitine slightly but steadily increased the oxidation rate (Fig. 1 D). The homogenization procedure was very important for the FAO assay. Strong homogenization decreased the oxidation rate and low activities were also recorded if the sample was minced with scissors only or tissue slices were used. Inhibition in oxidation rate was observed if homogenate was stored longer than 2 h at 0–4°C.

Assay of enzymatic activities

The homogenates of the whole MQF (but not predominantly red or white parts) were further homogenized for 1 min and used for enzyme assays. Cytochrome oxidase activity (CytOx) (E.C. 1.9.3.1), for estimating the oxidative capacity of the muscle, was determined polarographically (Wherrett 1968) using Clark-electrode. Into the reaction chamber 3.0 ml 50 mM phosphate buffer (pH ..., temperature 78°C) and 100 μl homogenate were added. After few minutes 50 μl of a mixture of 60 ... N N N',N' tetramethylphenylenediamine and 120 mM sodium ascorbate was added and the blank reaction registered for few minutes. The reaction was started by the addition of 10 μl 4 mM cytochrome c ... registered for approx. 5 min.



. The differences in the fatty acid oxidation capacity (FAO) and the activities of cytochrome (CytOx), malate dehydrogenase (MDH), triosephosphate dehydrogenase (TPDH) and lactate rogenase (LDH) between the grossly hite (white columns) and grossly red (black columns) parts QF of untrained NMRI mice in EXP III The figures below show the t-value for the difference, for the significance of the difference and per cent difference in the activities between hite and uscle tissue types

I III. EXP III The means and standard deviations of different ariables in control and trained animals together with the per cent differences between groups and the significance of the difference. FAO and enzyme activities are referred per muscle wet weight.

able	Control (n = 14)	Trained (= 13)	%	p
ht of animals	38 6 ± 1 7	36 6 ± 1.8	− 5 2	< .05
ht of MQF	256 ± 24	244 ± 23	− 4.7	n.s.
O white part	17.2 ± 4 3	19 3 ± 6 6	+ 12.3	s.
O red part	65 6 ± 13 8	80 8 ± 13 6	+ 23 2	< .01
O MQF	41 7 ± 8 4	51.8 ± 14 9	+ 24 2	< .05
Ox white part	8 4 ± 2 6	10.7 ± 3 2	+ 27.2	< .05
Ox red part	17 7 ± 4 2	23 8 ± 6 6	+ 34 5	.01
Ox MQF	18 0 ± 4 2	23.8 ± 7 1	+ 31 7	< .05
H white part	132 ± 14	164 ± 33	+ 24 2	.01
H red part	236 ± 22	289 ± 36	+ 22.5	< .001
H MQF	171 ± 16	222 ± 28	+ 29 8	.001
DH white part	99 ± 19	111 ± 27	+ 12.1	s.
DH red part	115 ± 25	116 ± 17	+ 1 7	s.
DH MQF	103 ± 21	111 ± 23	+ 7 8	s.
H white part	346 ± 41	357 ± 55	+ 3.2	s.
H red part	326 ± 40	299 ± 53	− 8.3	
H MQF	319 ± 39	314 ± 45	− 1 6	

Malate dehydrogenase and triosephosphate dehydrogenase activities are expressed as nmol/min/mg uscle wet weight at 22°C.

TABLE II EXP II The means and standard deviations of different variables in control and [trained] animals together with the per cent differences between the groups and the significance of the difference.

Variable	Control (n = 27)	Trained (n = 35)	%	p
Weight of animals	29.1 ± 1.9	29.7 ± 2.5	+ 1.9	n.s.
Weight of MQF	228 ± 19	236 ± 26	+ 3.7	n.s.
Protein content of MQF	193 ± 21	189 ± 2.	− 2.0	n.s.
FAO/muscle wet weight	41.9 ± 6.6	57.2 ± 11.1	+ 36.5	< .001
FAO/muscle protein	220 ± 46	308 ± 81	+ 40.0	< .001
CytOx/muscle wet weight[a]	18.0 ± .9	3.6 ± 5.2	+ 30.6	< .001
CytOx/muscle protein[a]	94.0 ± 16.8	125.4 ± 28.4	+ 33.4	< .001
LDH/muscle wet weight[b]	286 ± 58	256 ± 51	− 10.5	< .05
LDH/muscle protein[b]	1 496 ± 345	1 366 ± 300	− 8.7	n.s.

[a] Cytochrome c oxidase activity as nmol O consumed/min/mg at 28°C.
[b] Lactate dehydrogenase activity as nmol NADH oxidized/min/mg at 22°C.

($p < 0.001$) activities increased slightly more (31.7 and 29.8%) than FAO. Endurance t[raining] had no significant effects on the anaerobic (LDH) or glycolytic (TPDH) capacities (Ta[ble]

Differences in FAO and enzyme activities between grossly red and grossly white muscle of untrained mice

The basic differences between white and red part of MQF of untrained mice in C[ytOx,] MDH, TPDH and LDH activities and in FAO are shown in Fig. 2. In the red part [FAO] was 4 times higher than in the white part (65.6 ± 13.8 and 17.2 ± 4.3 dpm/min/[mg wet] weight). CytOx and MDH activities in red tissue were twice as high as in the white [tissue] ($p < 0.001$) whereas the differences in TPDH or LDH activities were not significan[t].

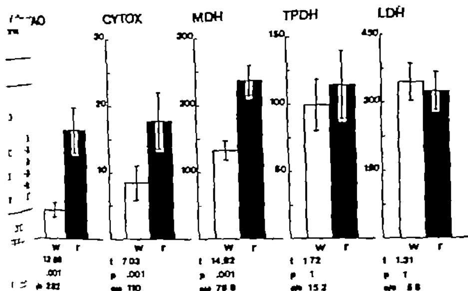
Effects of endurance training in grossly red and grossly white muscle tissue

The effects of endurance training on FAO and enzyme activities in red and white [tissue] and for comparison in the whole MQF (mixed) are given in Table III. The FAO i[n red] tissue increased more (23.2%) than in white tissue (12.3%). In absolute terms the FA[O in] red muscle increased almost seven times more than in white tissue. CytOx and [MDH] activities increased more in red tissue although the per cent change was rather similar i[n both] types. No clear effects of training were registered in the activities of TPDH or LDH.

The relationships between FAO and enzyme activities

The intercorrelations between FAO, CytOx and LDH in EXP II are given in Tabl[e IV.] FAO correlated positively and highly significantly ($p < 0.001$) with CytOx. In traine[d and] untrained groups, separately, the correlation coefficients were lower. Correlations be[tween] anaerobic capacity (LDH) and aerobic (FAO or CytOx) were more random, most of [them] were negative.

The intercorrelations of FAO, CytOx, MDH, TPDH and LDH in EXP III are giv[en in] Table V for the both muscle types and for all the observations. The strongest correla[tions] were observed between variables of oxidative metabolism (CytOx and M[DH]



2 The differences in the fatty acid oxidation capacity (FAO) and the activities of cytochrome ... (CytOx), malate dehydrogenase (MDH), triosephosphate dehydrogenase (TPDH) and lactate ... (LDH) between the grossly white (white columns) and grossly red (black columns) parts ... of untrained NMRI mice in EXP. III. The figures below show the t-value for the difference, ... for the significance of the difference and per cent difference in the activities between white and ... muscle tissue types.

... III EXP III The means and standard deviations of different variables in control and trained animals together with the per cent differences between groups and the significance of the difference. FAO and enzyme activities are referred per muscle wet weight.

...ble	Control (n = 14)	Trained (−13)		p
...ht of animals	38.6 ± 1.7	36.6 ± 1.8	− 5.2	< .05
...ht of MQF	256 ± 24	244 ± 23	− 4.7	n.s.
...O white part	17.2 ± 4.3	19.3 ± 6.6	+ 12.3	n.
...O red part	65.6 ± 13.8	80.8 ± 13.6	+ 23.2	< .01
...O MQF	41.7 ± 8.4	51.8 ± 14.9	+ 24.2	< .05
...Ox white part	8.4 ± 2.6	10.7 ± 3.2	+ 27.2	< .05
...Ox red part	17.7 ± 4.2	23.8 ± 6.6	34.5	< .01
...Ox MQF	18.0 ± 4.2	23.8 ± 7.1	+ 31.7	< .05
...H white part	132 ± 14	164 ± 33	+ 24.2	< .01
...H red part	236 ± 22	289 ± 36	+ 22.5	.001
...H MQF	171 ± 16	222 ± 28	+ 29.8	< .001
...DH* white part	99 ± 19	111 ± 27	+ 12.1	n.
...DH red part	113 ± 25	116 ± 17	+ 1.7	n.
...DH MQF	103 ± 21	111 ± 23	+ 7.8	
...H white part	346 ± 41	357 ± 55	+ 3.2	n.
...H red part	326 ± 40	299 ± 53	− 8.3	
...H MQF	319 ± 39	314 ± 45	− 1.6	

...ate dehydrogenase and triosephosphate dehydrogenase activities are expressed as nmol/min/mg ... wet weight at 22°C.

TABLE IV Correlation coefficients between the measured variables in EXP II. The significant correlation coefficients $p < 0.05$, $p < 0.01$ and $p < 0.001$

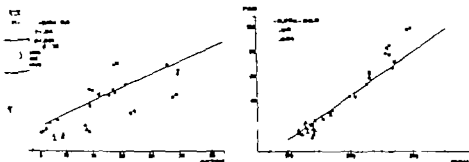
Group	CytOx/FAO	LDH/FAO	LDH/CytOx
Wet weight			
Controls	.026	.203	− 190
Trained	.231	−.244	−.327
Together	464	−.237	− 164
Protein			
Controls	.354	.503	050
Trained	.406	163	− 130
Together	.576	106	− 164

especially if all measured values from the different muscle samples are accounted (Fig. [illegible]). Correlation coefficients were then statistically highly significant.

Another positive correlation pattern existed between TPDH and LDH activity ($p < 0.001$). In this case correlation coefficients were high also in separate muscle types (Table V). The relationships between TPDH and oxidative variables were [illegible] mostly positive. On the other hand, LDH activity correlated often negatively with oxidative variables. These correlations were statistically significant only in some cases.

TABLE V Correlation coefficients between the measured variables in EXP III in different muscle types separately in control and trained groups and the whole sample. Statistical significances as in Table IV

Muscle group	CytOx/FAO	MDH/FAO	TPDH/FAO	LDH/FAO	MDH/CytOx	TPDH/CytOx	LDH/CytOx	TPDH/MDH	LDH/MDH	LDH/TPDH
White										
Controls	.342	.037	− 026	−.262	.306	.211	164	.080	.011	[illegible]
Trained	189	.325	.201	062	.676	.240	775	−.362	409	[illegible]
Together	.293	.318	178	−.024	640*	.305	.543	−.072	.317	[illegible]
Red										
Controls	.077	719	165	−.097	− 153	.026	−.336	428	.313	[illegible]
Trained	.098	− 142	.316	.371	790*	217	430	.045	.599*	[illegible]
Together	.310	.474	.208	−.00[illegible]	672	133	.008	195	146	[illegible]
Whole muscle										
Controls	.257	164	−.055	− 062	.402	179	.249	.094	[illegible]	[illegible]
Trained	137	.026	.270	127	.857	400	.611	124	.506	[illegible]
Together	.326	.353	.203	.045	777*	.362	.397	.210	.270	[illegible]
All values										
Controls	.643	.907	[illegible]85	−.230	.580*	.230	−.214	.359	−.083	[illegible]
Trained	.613	774	177	−.306	795	.224	.003	.035	− 122	[illegible]
Together	638	.815	.244	−.282	751	164	−.089	.219*	− 129	[illegible]



3. The correlation between fatty acid oxidation capacity (FAO) and the activities of cytochrome ... (CytOx) and malate dehydrogenase (MDH) in the different parts of MQF and the whole muscle of ... mice in EXP III.

Discussion

... largest differences in the properties of red and white tissue samples were recorded in ...dative capacity as expected. Red muscle oxidized fatty acids approx. 4 times faster than ... tissue. CytOx and MDH activities were twice as high in red than in white samples. ...dwin *et al.* (1972) have obtained similar results using the superficial, white and deep, red ...tions of MQF of rat. In rat the differences were, however much more pronounced, fatty ...d oxidizing capacity and palmityltransferase activity were approx. seven times, CytOx ...hly 5 and citrate synthase activity 3 times higher in the red than in the white tissue. The ...son for the greater differences in rat is maybe caused by the sample because from rat it is ...sible to get the red and the white muscle in a more representative form. In addition, ...cies differences may exist.

The red type of muscle is thus characterized by high oxidative capacity. Pette (1966) has ...orted that in different kinds of muscles of rabbit the capacities of the pathways for ...idative energy metabolism occur in rather constant ratios. This kind of constancy in ...zyme activity ratios probably provides only quantitative differences in the amount of ...tochondria in red and white tissues. In the present study in agreement with the results of ...dwin *et al.* (1972) it was shown that the differences in the activities of citric acid cycle ...d respiratory chain enzymes between red and white muscle were less than in fatty acid ...idation capacity. Baldwin *et al.* (1972) and Beatty *et al.* (1972) have further observed that ...e difference in FAO is markedly greater than in the capacity to oxidize pyruvate. All ...ese observations suggest that different pathways for oxidative energy production do not ...pear in exactly constant ratios in the red and white muscle types of mouse and rat. On the ...ntrary they show that the FAO in red tissue is more pronounced. The present results ...directly give support to the hypothesis that there are also qualitative differences between ...e mitochondria in red and white tissues as observed by Pande and Blanchaer (1971). The ...tochondria in red muscle are more specialized for f tty acid oxidation.

No clear differences in anaerobic or glycolytic capacities were observed between red and ...hite muscle types. The reason for this might be that in mice the red tissue is fast contracting ...hich is characteristic of rodents (Barnard *et al.* 1971 Baldwin *et al.* 1973). For fast

TABLE IV Correlation coefficients between the measured variables in EXP II. The significance of correlation coefficients. $p < 0.05$, $p < 0.01$ and $p < 0.001$

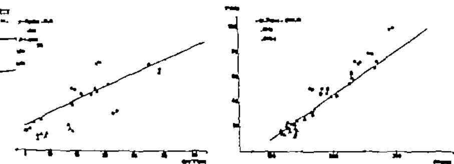
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especially if all measured values from the different muscle samples are accounted (Fig. 3). Correlation coefficients were then statistically highly significant.

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TABLE V Correlation coefficients between the measured variables in EXP III in different muscle types separately in control and trained groups and the whole sample. Statistical significance as in Table IV

Muscle group	CytOx/ FAO	MDH/ FAO	TPDH/ FAO	LDH/ FAO	MDH/ CytOx	TPDH/ CytOx	LDH/ CytOx	TPDH/ MDH	LDH/ MDH	LDH/ TPDH
White										
Controls	.342	.037	−.026	−.262	.306	.211	164	.080	.011	[illegible]
Trained	189	.325	.201	.062	676	.240	775	−.362	409	[illegible]
Together	.293	.318	178	− 024	640	305	.543	−.072	.317	[illegible]
Red										
Controls	.077	719	165	−.097	− 153	.026	−.336	428	.313	[illegible]
Trained	.098	− 142	.316	371	790*	.217	.430	.045	.559*	[illegible]
Together	.310	474	.208	−.002	.672	133	.008	195	146	[illegible]
Whole muscle										
Controls	.257	164	− 055	−.062	402	179	.249	.094	.324	[illegible]
Trained	137	.026	.270	127	.857	400	611	124	.586	[illegible]
Together	.326	.353	.203	.045	777	.362	.397*	.210	.270	[illegible]
All values										
Controls	.643	.907	.285	−.230	.580*	.230	−.214	.359*	−.083	[illegible]
Trained	613	774	177	−.306	795	.24	.003	.035	− 122	[illegible]
Together	638*	.815	.44	−.8	751	164	−.089	.219*	− 129	[illegible]



1. The correlations between fatty acid oxidation capacity (FAO) and the activities of cytochrome ...se (CytOx) and malate dehydrogenase (MDH) in the different parts of MQF and the whole muscle of ...ll mice in EXP. III

Discussion

...largest differences in the properties of red and white tissue samples were recorded in ...ative capacity as expected. Red muscle oxidized fatty acids approx. 4 times faster than ...te tissue. CytOx and MDH activities were twice as high in red than in white samples. ...dwin *et al.* (1972) have obtained similar results using the superficial, white and deep, red ...tions of MQF of rat. In rat the differences were, however much more pronounced; fatty ...d oxidizing capacity and palmityltransferase activity were approx. seven times, CytOx ...ivity 5, and citrate synthase activity 3 times higher in the red than in the white tissue. The ...son for the greater differences in rat is maybe caused by the sample because from rat it is ...sible to get the red and the white muscle in a more representative form. In addition, ...cies differences may exist.

The red type of muscle is thus characterized by high oxidative capacity. Pette (1966) has ...orted that in different kinds of muscles of rabbit the capacities of the pathways for ...idative energy metabolism occur in rather constant ratios. This kind of constancy in ...zyme activity ratios probably provides only quantitative differences in the amount of ...tochondria in red and white tissues. In the present study in agreement with the results of ...ldwin *et al.* (1972) it was shown that the differences in the activities of citric acid cycle ...d respiratory chain enzymes between red and white muscle were less than in fatty acid ...idation capacity. Baldwin *et al.* (1972) and Beatty *et al.* (1972) have further observed that ...e difference in FAO is markedly greater than in the capacity to oxidize pyruvate. All ...ese observations suggest that different pathways for oxidative energy production do not ...ppear in exactly constant ratios in the red and white muscle types of mouse and rat. On the ...ntrary they show that the FAO in red tissue is more pronounced. The present results ...directly give support to the hypothesis that there are also qualitative differences between ...e mitochondria in red and white tissues as observed by Pande and Blanchaer (1971). The ...itochondria in red muscle are more specialized for fatty acid oxidation.

No clear differences in anaerobic or glycolytic capacities were observed between red and ...hite muscle types. The reason for this might be that in mice the red tissue is fast contracting ...which is characteristic of rodents (Barnard *et al.* 1971, Baldwin *et al.* 1973). For fast

contractions, high glycolytic and anaerobic capacities are essential. The differences in these [illegible] capacities in MQF of rat were slightly higher (Baldwin *et al* 1973) than observed in this study [illegible]

It has been repeatedly observed that endurance type of training increases the oxidative [illegible] capacity of muscle (*e.g.* Holloszy 1967 Mole 1971 Baldwin *et al* 1972, Gollnick *et al* [illegible] 1973 Kiessling *et al* 1974) The activities of enzymes of citric acid cycle, respiratory chain [illegible] and β-oxidation are increased (Holloszy *et al* 1971 Mole *et al* 1971 Morgan *et al* 1971 [illegible] as are the capacities to oxidize fatty acids or carbohydrates (Baldwin *et al* 1972, Mole and [illegible] Holloszy 1970) In the present study most pronounced increase in FAO was observed in EXP II with F -hybrids. This increase was 36.5% while in EXP I and EXP III the increase [illegible] were on the average 24 %. According to Mole *et al* (1971) the FAO in MQF and gastrocnemius-muscles of rat was almost doubled due to endurance training. In the above mentioned study the training of rats was very intensive and that may be the reason for the large increase. The physical inactivity of rats when compared to mice in cage conditions may also influence the magnitude of the increase.

The increase in CytOx and MDH activities was on the average 30%. The increase of FAO in EXP II was higher than in EXP I and EXP III although the increase in CytOx activity in EXP II and EXP III was of the same magnitude. This observation suggests that the training type which provides greater endurance might more strongly adapt β-oxidative capacity Increased β-oxidative capacity has a sparing effect on muscle glycogen stores [illegible] it decreases blood glucose consumption thus preventing fatigue during submaximal loading (Keul *et al* 1972).

Results regarding the effects of endurance training in different muscle types are contradictory Histochemical studies suggest that the oxidative capacity increases primarily in white fibers (Gollnick *et al* 1973 Zika *et al* 1973) Biochemical data show that endurance training adapts the oxidative capacity of red muscle more than that of white muscle (Baldwin *et al* 1972) The present results show that the relative increase in oxidative capacity in both muscle types is approximately similar but the absolute change in red tissue is manyfold when compared to white tissue. Both FAO and the activities of CytOx and MDH increased clearly more in the red proximal part of mouse MQF than in the distal white part. The difference in changes was greater in FAO than in CytOx or MDH activities These observations suggest that either the white muscle tissue is weaker in its adaptive capacity than red muscle or the training affected the white fibers less than the red fibers.

The adaptations caused by training must always be revised against the intensity and duration of training. The training program used by Baldwin *et al* (1973) decreased very strongly the glycogen content in the red part of the thigh muscle of rat whereas it had practically no effects in the white part Thus it is evident that white muscle cells contracted irregularly during the loading. The recruitment order of motor neurons is determined primarily according to the size principle (Granit 1970 Millner Brown *et al* 1972) The small-sized slowly conductive motor neurons are activated first and they recruit oxidative fibers. When the level of tension increases also large motor units, which are composed of white fibers, are recruited. The increasing tension is achieved at lower tension levels by recruiting "new" motor units whereas if the tension still increases additional muscular power is mainly produced by higher impulse frequency (Gydikov and Kosarov 197 Millner Brown *et al*

[illegible]). It is generally accepted that impulse frequency has regulating role also on the [illegible]abolic properties of muscle cells (Guth 1968, Cotter *et al.* 1973). Thus red muscle cells [illegible]ctioning at higher frequency level during light loading are probably more affected by [illegible]latory adaptive responses. This might explain the higher increase in the oxidative [illegible]acity in red muscle after training programs used in this study.

[illegible]he evaluation of the adaptive capacity of different muscle types is difficult on the basis [illegible] one experiment series. I[illegible] the present study a possibility exists that the loading level [illegible]lied activated the motor units in white tissue differently causing a different increase in [illegible] oxidative capacity of separate motor units. On the other hand, it is not known what is [illegible] relationship in the activation between the white surface part or the distal part of the [illegible] muscle. The effect of endurance training in the white distal part of NMRI-mice [illegible] (P III) [illegible]as rather small when compared to changes observed in rats (Baldwin *et al.* [illegible]72). The reason for this is probably the lighter loading level used in the present study. [illegible] muscle fibers are probably rather adaptive, because Cotter *et al.* (1973) have observed [illegible]t low-level electrical stimulation of white muscle tissue can increase the capillary density [illegible] citrate synthase activity close to the level recorded in red tissue already after a few [illegible]eks. However it must be emphasized that this effect provided continuous activation of [illegible] [illegible] fibers.

In the present study it was observed that endurance training increased the oxidative [illegible] energy producing capacity in the red and the white muscle in almost constant ratio. Thus [illegible] homogeneous adaptation of oxidative processes does not change the cell-type distribu[illegible] but increases the basic level of oxidative metabolism in both fiber types. Endurance [illegible]ning had only slight effects on the glycolytic or anaerobic capacities in the muscle types [illegible]died. The adaptive effects of endurance training are thus mainly directed to the oxidative [illegible] energy metabolism and are qualitatively to a high extent parallel in the red and in the [illegible] muscle tissue.

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From the Institute of Physiology University of Lund, Sweden

Difference in Sensitivity of Parotid Glands brought about by Disuse and Overuse

By

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Abstract

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...nsitivity to methacholine of the parotid glands in rats maintained on liquid diet (disuse at sensitiza... period of 2 weeks or 3–4 weeks was compared with that of the parotid glands in rats on pelleted diet (overuse at desensitization). In the rats on the liquid diet, was found that the dose needed to ... just perceptible secretion of saliva was smaller, that the secretion started earlier and continued ... longer time period, and further that the amount of saliva secreted expressed per gland ... mg in ... nse to submaximal doses of the sialogogue drug was bigger when compared with the rats on the ... ted bulk diet. These findings are interpreted as signs of higher degree of sensitivity of the glands in ... ats on the liquid diet than of those in the rats on the pelleted bulk diet.

...ts fed on a liquid diet the parotid glands were found to loose weight, and the acetyl... line synthesizing capacity of the intraglandular cholinergic nerves decreased. These ... ts were thought to be consequences of reduced reflex stimulation of the glands (Ek... 1973). In these experiments the sensitivity of the secretory cells to chemical stimuli ... also investigated since decreasing the flow of secretory impulses, for instance by cutting ... rent fibres of the secretory reflex arc, has been shown to cause a supersensitivity in salivary ... ds (see Emmelin 1952, 1961 a, 1967). There was some indication that a supersensitivity ... ht develop in parotid glands of rats on liquid diet, but when comparisons were made ... h rats on an ordinary pelleted diet no statistically significant differences appeared.

...n the present expts. rats on liquid diet were compared with litter mates fed on a bulky ... t, supposed to increase the reflex stimulation of the glandular cells. Previous experiments ... e shown that the weight and the acetylcholine forming capacity of parotid glands increase ... en rats are given a cellulose rich diet (Ekström 1974).

Methods

...ale rats of Sprague-Dawley strain bred at this Institute were used. The rats were 6–11 months old ... the expts. started. They were either given liquid diet, prepared twice day by mixing 2 parts of ... er with 1 part of powder of standard diet for rats (R 3, Astra-E os), or pelleted bulk diet made

from the same standard diet but containing 30% cell lose instead of the usual 3 the rats given the liquid diet were litter mates to those given the bulk diet The diets and water were given *ad libitum*. The rats on the liquid diet were placed in cages with bottoms of wire-netting, instead of bottoms covered by saw shavings, this was done in order to further reduce their opportunity to gnaw At the start and at the end of the experimental period, the body weights were determined After an experimental period of 2 weeks or of 3–4 weeks, the animals were anesthetized with chloralose (100 mg/kg) i.v. after ether induction. The parotid duct on the right side was exposed near the entrance in the mouth through a skin incision and cannulated with a fine glass cannula, which gave 94 drops from 1 ml distilled water. Saliva appearing at the tip of the cannula was collected on filter paper and weighed. To estimate the threshold dose and the maximal secretory responses a series of doses of methacholine chloride (0 05–10 μg/kg) ... The amount of saliva secreted was expressed in mg per gland or in mg saliva per mg gland weight (dry wt). To estimate the maximal secretory response, as described by Schneyer and Hall (1966) pilocarpine hydrochloride (10 mg/kg) was injected i.p In this type of expt. drops of saliva were recorded ... period of 15 min on a smoked drum with an ordinate writer The maximal response as expressed in saliva per min per gland or per min per mg gland weight (dry weight). The two litter mates to be compared one on the liquid diet and one on the pelleted bulk diet, were always tested consecutively At the end of the expt the rats were killed by i v injection of air and the parotid gland which had been examined for secretory response was carefully dissected out cleaned and weighed before (wet weight) and after heating to 105–110°C for 48 h (dry weight). From rats studied over a period of 3–4 weeks the parotid glands on the left side which was not cannulated were also removed cleaned and prepared for ... of the choline acetyltransferase activity using the method of Catherine Hebb (Nordenfelt 1967) The enzyme activity was expressed in μg acetylcholine chloride formed per hour per pool of glands (total ...) and in μg acetylcholine chloride formed per hour per g acetone powder (concentration). Further technical details are given in the text.

For statistical analysis Students t test was used paired comparisons were made between the rat given the liquid diet and its litter mate given the pelleted bulk diet. P-values less than 0 05 were considered significant

Results

Body weights At the start of the expts there were no differences in the weights between the rats to be given the liquid diet and those to be given the pelleted bulk diet In the series of 2 weeks the body weights of the rats on the liquid diet increased ($p < 0\,001$) from (mean ± S E.) 350 ± 9 to 361 ± 7 g (n = 20) *i.e* by about 3%, while that of the rats on the pelleted bulk diet remained unchanged (from 353 ± 8 to 354 ± 7 g, n = 20). In the series of 3–4 weeks there was also an increase ($p < 0\,05$) in body weights of the rats on the liquid diet from 377 ± ... to 386 ± 5 g (n = 17), *i e* by about 2%, while the weights of the rats on the pelleted bulk diet did not change (from 377 ± 5 to 380 ± 5 g, n = 17)

Gland weights As shown in Table I the wet weights of the parotid glands in the rats on the liquid diet, both in the series of 2 weeks and in the series of 3–4 weeks, were about 50% of those in the rats on the pelleted bulk diet. The dry weights were similarly affected

TABLE I Gland weights (wet and dry) of parotid glands in rats given liquid diet and in rats given pelleted bulk diet for 2 weeks and 3–4 weeks. Values are mean ± S E. In brackets are numbers of observations.

		Wet weights (mg)	Liquid/pelleted ( )	Dry weights (mg)	Liquid/pelleted (%)
2 weeks	Liquid diet	90 6 ± 3 1 (20)	47 7 ± 2.2 (20)	6 7 ± 0.9 (20)	49 ... (20)
	Pelleted bulk diet	194 2 ± 7 9 (20)	p 0 001	55.5 ± 2.4 (20)	p 0 001
3–4 weeks	Liquid diet	99 7 ± 5.4 (17)	48 2 ± 1 1 (17)	30.9 ± 1 9 (17)	46.3 ± 3 1 (17)
	Pelleted bulk diet	218 4 ± 9.. (17)	p 0 001	67 6 ± 2 9 (17)	p 0 001

II The secretory responses of parotid glands in mg saliva after 2, 5 and 10 μg/kg I. of methacholine in rats given liquid diet and in rats given pelleted bulk diet for 2 and 3–4 eeks. Values are mean ± S.E. In brackets are numbers of observations

		2 μg/kg		5 μg/kg		10 μg/kg	
ks	Liquid diet	5.7±0.9 (15)	p>0.1	9.5±0.9 (15)	p>0.1	17.9±1.5 (15)	p<0.1
	Pelleted bulk diet	6.6±1.0 (15)		10.8±1.0 (15)		21.2±1.8 (15)	
eeks	Liquid diet	2.7±0.4 (10)	p<0.02	6.3±0.5 (10)	p<0.001	14.7±1.5 (10)	p<0.001
	Pelleted bulk diet	4.2±0.5 (10)		10.0±0.8 (10)		24.2±2.4 (10)	

line acetyltransferase activity The amount of acetylcholine formed during 1 h by a of 11 parotid glands from rats fed on the liquid diet over the period of 3–4 weeks was 3 μg, while the pool of 11 glands from rats fed on the pelleted bulk diet over the same od of time formed 317.5 μg, i.e. the total enzyme activity was about 30% less in the otid glands of the rats on the liquid diet. As to the enzyme concentration (per g acetone der) it was 841 μg acetylcholine in the glands of the rats on the liquid diet and 560 μg lcholine in the rats on the pelleted bulk diet the fact that the enzyme concentration was er in the rats on the liquid diet is attributed to the pronounced difference in gland ht between the two groups of animals.

cretory threshold doses. When the two litter mates were compared, one given the liquid and the other the pelleted bulk diet, it was found that the amount of methacholine ded to evoke a just perceptible secretion of saliva from the parotid gland was the same omewhat less for the rat on the liquid diet. In the series of 2 weeks the mean threshold e was 0.34±0.08 μg/kg for 15 rats on the liquid diet and for the corresponding 15 rats he pelleted bulk diet 0.49±0.09 μg/kg. In the series of 3–4 weeks the dose was 0.80±0.08 kg for 10 rats given the liquid diet and 1.0±0.0 μg/kg for the corresponding 10 rats n the pelleted bulk diet. On both occasions the difference between the two groups of mals was significant at the p<0.05 level.

ecretory responses to some standard doses. The amount of saliva secreted in response to 5 and 10 μg/kg of methacholine is shown in Table II and III In Table III the saliva is ted to the gland weight. In the series of 2 weeks the responses of the glands in the rats on the liquid diet were about the same as those of the rats fed on the pelleted bulk diet

LE III The secretory response of parotid glands in mg saliva per mg tissue after 2, 5 and 10 μg/kg I. of methacholine in rats given liquid diet and in rats given pelleted bulk diet for 2 weeks and 3–4 weeks. Values are mean ± S.E. In brackets are numbers of observations

		2 μg/kg	5 μg/kg	10 μg/kg
weeks	Liquid diet	0.2183±0.0330 (15)	0.3617±0.0378 (15)	0.6221±0.0649 (15)
		p 0.001	p 0.001	p<0.001
	Pelleted bulk diet	0.1232±0.0180 (15)	0.2005±0.0208 (15)	0.3904±0.0355 (15)
4 weeks	Liquid diet	0.0929±0.0156 (10)	0.2161±0.0156 (10)	0.4934±0.0536 (10)
		p 0.1	p<0.05	p<0.05
	Pelleted bulk diet	0.0623±0.0065 (10)	0.1500±0.0136 (10)	0.3608±0.0375 (10)

from the same standard diet but containing 30% cellulose instead of the usual 3 ,., the rats given the liquid diet were l tter mates to those given the bulk diet. The diets and water were given *ad libitum* The rats on the liquid diet were placed in cages with bottoms of wire-netting, instead of bottoms covered by wood shavings this was done in order to further reduce their opportunity to gnaw At the start and at the end of the experimental period, the body-weights were determined. After an experimental period of 2 weeks or of 3–4 weeks, the a imals were anesthetized with chloralose (100 mg/kg) i v after ether induction The parotid duct on the right side was exposed near the entrance in the mouth through a skin incision and cannulated with a fine glass cannula, which gave 94 drops from 1 ml distilled ater Saliva appearing at the tip of the cannula was collected on filter paper and weighed. To estimate the threshold dose and the maximal secretory responses a series of doses of methacholine chloride (0 05–10 µg kg) as given The amount of saliva secreted was expressed in mg per gland or in mg saliva per mg gland eight (dry weight). To estimate the maximal secretory response, as described by Schneyer and Hall (1966) pilocarpine hydrochloride (10 mg/kg) was injected i.p In this type of expt. drops of saliva were recorded each over a period of 15 min on a smoked drum with an ordinate writer The maximal response as expressed as saliva per min per gland or per min per mg gland weight (dry weight). The two litter mates to be compared, one on the liquid diet and one on the pelleted bulk diet, were always tested consecutively At the end of the expt. the rats were killed by i. i jection of air and the parotid gland which had been examined for secretory response was carefully dissected out, cleaned and weighed before (wet eight) and after heating to 105–110°C for 48 h (dry weight) From rat studied over a period of 3–4 weeks the parotid glands from the left side which was not cannulated were also removed, cleaned and prepared for bioassay of the choline acetyltransferase activity using the method of Catherine Hebb (Nordenfelt 1963) The enzyme activity was expressed i µg acetylcholine chloride formed per hour per pool of glands (total activity) and i µg acetylcholine chloride formed per hour per g acetone powder (concentration). Further technical details are given in the text.

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3–4 weeks	Liquid diet	99 7 ± 5 4 (17)	48.2 ± 1 1 (17)	30 9 ± 1 9 (17)	46.3 ± 3 1 (17)
	Pelleted bulk diet	18 4 ± 9.2 (17)	p 0 001	67 6 ± 2.9 (17)	p 0 001



LIQUID DIET PELLETED BULK DIET

The maximal secretory response to pilocarpine, 10 mg/kg i.p. of the parotid gland of rat maintained liquid diet and of its litter mate maintained on pelleted bulk diet; the rats belonged to the series of 3–4 Records from above: minute marks, drops of saliva, signal, marking the start of the injection of arpine and the onset of secretion, salivary flow rate recorded with an ordinate writer. The time of of the secretion was in the rat on the liquid diet 25.1 and in the rat on the pelleted bulk diet 30.7 the injection of pilocarpine.

he liquid diet. In Fig. 1 the recordings from two litter mates, one on liquid diet and the r on pelleted bulk diet, are shown after the injection of pilocarpine; in these recordings time of onset of secretion was also marked.

hen expressed in mg saliva per min and mg tissue, both series showed a bigger maximal onse (p 0.001) in the rats on the liquid diet. The figures in the series of 2 weeks were 0.10 (liquid diet) and 0.63 ±0.04 (pelleted bulk diet), and in the series of 3–4 weeks 1±0.09 and 0.76±0.05 respectively

Discussion

en the body-weights of the rats are considered it appears that the animals endured the well. As could be expected from the previous findings (Ekström 1973 1974) the weights, articular but also the acetylcholine forming capacity of the parotid glands were lower he rats maintained on the liquid diet than in those on the pelleted bulk diet.

n studies on sensitivity of salivary glands comparisons have usually been made between experimental gland and its fellow gland in one and the same animal or repeated observa- ns have been made over long periods of time on a gland (Emmelin 1964). A technique t allows repeated examinations of the parotid glands in rats has not been developed yet, attempt to reduce the number of variables was in the present study to make comparisons ween litter mates and further t examine the two litter mates on the same occasion. The ree of sensitivity of a gland to chemical stimuli may be expressed in terms of threshold e, time of onset and duration of secretion, and olume of saliva secreted in response to submaximal dose, as pointed out by Emmelin (1952). Sensitization is known to be most rked after postganglionic section of the main secretory nerve, the parasympathetic including not only the action on the gland cells of that fraction of the transmitter set free

when not related to the weights. In the series of 3–4 weeks the amount of saliva at all the doses, less in the animals on the liquid diet. When the amount of saliva was related to the gland weight it can be seen that in the series of 2 weeks the liquid diet secreted a bigger amount of saliva than those on the pelleted bulk diet doses tested. In the series of 3–4 weeks the amount of saliva was the same in the at the dose of 2 µg/kg and bigger in the rats on the liquid diet at the two larger III shows also that the differences in the amount of saliva secreted per gland bigger in the series of 2 weeks than in the series of 3–4 weeks, e.g when co figures at the dose of 5 µg/kg the amount of saliva secreted per gland weight in the the liquid diet was in percent of that in the rats on the pelleted bulk diet about l series of 2 weeks and in the series of 3–4 weeks about 145

Onset of secretion It was noticed that the animals on the liquid diet seemed to respond more promptly to an injection of methacholine than those on the other type of diet, when the time that elapsed between the beginning of the i.v injection of 5 µg/kg of the substance and the start of the secretion (i.e just when the meniscus of saliva began to move) was measured It was found that both in the series of 2 weeks and in the series of 3–4 weeks the rats on the liquid diet responded about 1 s earlier ($p < 0.01$). In the series of 2 weeks the figures were 6.2 ± 0.4 s ($n = 9$) for the rats on the liquid diet and 7.4 ± 0.4 s ($n = 9$) for the rats on the pelleted bulk diet and in the series of 3–4 weeks 6.7 ± 0.1 s ($n = 8$) and 7.7 ± 0.1 ($n = 8$) respectively

Duration of secretion A striking feature was that the secretion of the rats on the liquid diet continued over a longer period of time than that of the rats on the pelleted bulk diet. The time of secretion was determined using the dose of 5 µg/kg methacholine. In this type of expt care was taken to remove saliva appearing at the tip of the cannula frequently so as to make it easier to observe the end of the secretion The duration of the secretion was about twice as long ($p < 0.001$) in the animals on the liquid diet In the series of 2 weeks the mean duration was 128 ± 8 s in 10 rats on the liquid diet and 66 ± 4 s in 10 rats on the other type of diet in the series of 3–4 weeks the figures were 114 ± 8 s ($n = 9$) and 69 ± 3 s ($n = 9$) respectively

Maximal secretory response In both the series of 2 weeks and that of 3–4 weeks the maximal secretory response to pilocarpine of the parotid glands, expressed in mg saliva per min was found to be less ($p < 0.02$) in the rats on the liquid diet than in those on the pelleted bulk diet In the series of 2 weeks the mean response was, in 5 rats given the liquid diet 26.1 ± 1.8 and in the corresponding 5 rats on the pelleted bulk diet 34.2 ± 1.1 i.e the response after the liquid regime was in percent of that after the pelleted bulk regime 76.6 ± 5.4 ($n = 5$) In the series of 3–4 weeks the mean response in 6 rats on the liquid diet was 30.9 ± 2.1 and in the 6 rats on the pelleted bulk diet 41.8 ± 2.5 the percentage figure obtained was very similar to that of the series of 2 weeks, being 75.1 ± 6.1 ($n = 6$) It appeared from the tracings in this type of expt that the maximal secretory response was reached earlier in the rats on the liquid diet and further that the rate of secretion in these rats was not maintained at a high level for such a long time as in those on the pelleted bulk diet Although the i.p. injection of pilocarpine could not be as standardized as the i.v injection of methacholine it may be mentioned that the onset of secretion to pilocarpine wa [illegible] lly earlier in the [illegible]

arotid glands of the rats on the liquid diet had a maximal secretory capacity that was t 25 percent less than that of the rats on the pelleted bulk diet both in the series of 2 s and in the series of 3–4 weeks. The maximal secretory capacity of a salivary gland is n to be reduced by parasympathetic denervation or decentralization, and this is prob- connected to the glandular atrophy after these operations (Emmelin, Malm and ablad 1960).

seems reasonable to conclude from the present expts. that a difference in sensitivity of arotid glands in rats supplied with an intact innervation can be brought about by the f an experimental set up favouring sensitization of the gland cells (liquid diet) on one and desensitization (pelleted bulk diet) on the other hand as to desensitization, it is n that prolonged treatment with sialogogue drugs can depress the sensitivity of the l cells (Emmelin 1952).

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by nerve impulses but also that fraction of the transmitter continuously leaking from the nerve endings, see Emmelin 1967). It is less marked after section of the preganglionic nerve and even less after cutting afferent nerves from the tongue (Emmelin 1961 b). From these observations it appears to follow that the difference in sensitivity that may be expected to be at hand in the present experiments using animals with intact nervous pathways is small. Nevertheless, the present findings of lower threshold dose to methacholine, and of earlier start and longer duration of secretion in response to this substance in the rats given the liquid diet when compared with the rats on the pelleted bulk diet seem to indicate that the parotid glands of the rats on the liquid diet had a higher degree of sensitivity than those of the rats on the pelleted bulk diet. Such an idea is further supported by the results of the experiments in which pilocarpine was used: the secretion both started (at least in most cases) and reached the maximal flow rate earlier in the rats on the liquid diet than in the rats on the pelleted bulk diet. The glands of the rats on the liquid diet appeared to be more exhaustible, since they only maintained a high flow rate for a short time: this is a phenomenon seen in connection with sensitization (Emmelin and Muren 1951).

Since the difference in threshold was relatively small it is of interest to note that in rats treated over a period of time with a ganglion blocking substance the dose of methacholine needed to evoke secretion of saliva from the parotids was half of that needed for the glands in untreated animals (Ekström and Lindmark, to be published): the pathway of the preganglionic parasympathetic nerves of the parotid glands in this species is unknown and therefore these nerves could not be cut. In the cat, the parasympathetically decentralized parotid gland had a threshold dose to acetylcholine that was also about half of that of the contralateral gland (Strömblad 1955).

The volume or amount of saliva secreted in response to some standard doses of methacholine was the same in the two groups of animals in the series of 2 weeks, and in the series of 3–4 weeks it was even smaller in the rats on the liquid diet than in those on the pelleted bulk diet. For comparisons it may be mentioned that after prolonged ganglion blockade the amount of saliva secreted to a dose of 5 μg/kg methacholine was about 20 per cent more than from the parotid glands in untreated rats (Ekström and Lindmark, to be published), whereas after a postganglionic parasympathetic denervation the amount of saliva secreted was about 3 times that of the contralateral gland in response to the same dose of methacholine (Alm and Ekström 1976). When the amount of saliva secreted to the submaximal doses of methacholine is related to the gland weight it is found that the gland of the rat on the liquid diet secreted more than the gland of the rat on the pelleted bulk diet, and this applies both for the series of 2 weeks and the series of 3–4 weeks. Because of the very big difference in gland weight between the rats on the liquid diet and those on the pelleted bulk diet, it does not seem relevant to use the response to submaximal doses of methacholine as a parameter of sensitivity without relating the response to the gland weight. The higher figures obtained from the rats on the liquid diet, when the comparison is based on the gland weights, are interpreted as another sign of a higher degree of sensitivity of the glands of these animals compared with those in the rats on the pelleted bulk diet. That the weight is of importance for the amount of saliva secreted is shown by the results of the experiments in which the maximal secretory response to an i.p. injection of pil[illegible] was determined.

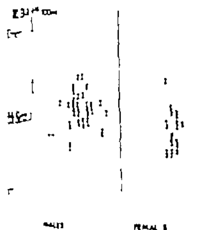


Fig. 1

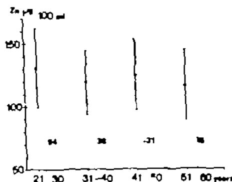


Fig. 2

1 Serum zinc levels of male and female blood donors from Oslo. Lines indicate mean levels ± S.D

2 Serum zinc levels in men at different ages. Mean ± S.D

... of age 20–40 years were recalled for further examinations because of their serum zinc levels ... were higher than the local mean in 19 men and lower than the mean in 21 men. Only 3 of them ... diagnosed disease. Subject number 24 (Table II) had an arthritis deformans, number 25 ... heart ... and number 36 (Table III) an arterial hypertension. They used regularly drugs according to their ... Blood samples of these 40 men were examined ... Technicon Autoanalyzer as ... rough ... of their condition.

... Blood samples were drawn at 8 ... after an overnight fast. Clotted samples were centrifuged ... serum ... used. For hormone determinations serum was conserved in − 20 C till the assays were ...

... The zinc determinations were performed using ... Perkin-Elmer Model 303 atomic absorption ... Standard solutions were prepared using stock solutions (Merck, Germany). The ... due to the difference in viscosity between the serum and the aqueous standard solutions was avoided ... dextran solution instead of water (Hackley *et al.* 1968). Standard solutions contained 154 ... of Na and Cl. The samples were undiluted, since optimal sensitivity could be obtained in the recorder ... in that range. Sources of contamination (rubber stoppers etc.) were excluded as far as possible ... non-haemolysed serum was used.

... follicle stimulating hormone (FSH) and serum luteinizing hormone (LH) were determined by ... double antibody radioimmunoassay (Reuter *et al.* 1973). The second antibody was fixed on ... cellulose according to the method of Wide *et al.* (1969). Kits by CEA-IRE-SORIN were used ... LER 907 ... used as reference preparation. Radioimmunoassay of serum testosterone was per... after extraction of 30 µl of serum in diethyl ether (ad 3.0 ml) as described by Dufau *et al.* 1972. ... the statistical treatment of the data regression analysis (Spearman ... correlation coefficient) and ... test were used.

Results

... zinc ... sex, age and height. The mean serum zinc level ± S.D. of the 154 male blood ... was 123.84 ... 28.49 µg/100 ml and that of the 95 females 111.36 ± 23.40 µg/100 ml. ... ranges were 73.5–220 in the men and 63.5–181.5 in the women (Fig. 1). The difference ... between the men and women was highly significant ($p < 0.001$). Fig. 2 shows mean serum ...

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From the Department of Physiology University of Oulu, Finland

Serum Testosterone Compared with Serum Zinc in Man

By

R. Hartoma

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Abstract

Hartoma, R. *Serum testosterone compared with serum zinc in man* Acta physiol. scand. [illegible] *101* 336-342.

Serum zinc was measured in 154 male and 95 female blood donors. Men had higher serum zinc levels [illegible] women, the difference being statistically significant ($p < 0.001$) The correlations between serum [illegible] serum testosterone, serum zinc and serum FSH and serum zinc and serum LH were studied in [illegible] aged [illegible]8-60 years and statistically significant positive correlation was found only between serum [illegible] and serum testosterone in men of age 36-60 years ($p < 0.005$). These results do not support the theory [illegible] slight zinc deficiency primarily effects pituitary gonadotropins but indicate that it would rather [illegible] on testicular level

There are very few observations of zinc deficiency in man. External features evidently [illegible] to zinc deficiency described by Prasad in a group of Iranian adult males were growth [illegible]tardation and marked hypogonadism (Prasad *et al* 1961), and Prasad also found [illegible] cases in Egypt (Prasad *et al* 1963) Therapy with zinc resulted in increased size of [illegible] external genitalia and the appearance of secondary sex characteristics in addition to [illegible] increase in height.

Zinc deficiency presents an extreme condition It was therefore interesting to [illegible] whether there is any relationship between zinc and pituitary-gonadal axis in normal [illegible] It seemed reasonable to estimate the mean level of serum zinc in the local population as the values stated in the literature differ (Kahn *et al* 1965 Prasad *et al* 1965). For comparison the serum zinc level was also measured in women Men whose zinc values deviated from the mean in either direction were selected for further investigation, including determination of serum testosterone, FSH and LH

Material and Methods

Subjects. Serum zinc levels were measured in 154 male and 95 female voluntary blood donors [illegible] spontaneously to the Blood Transfusion Service in Oulu during one summer month in 1975 These subjects were presumed to represent the healthy population in this town. Forms with questions about the personal data, weight and height, diseases and use of drugs were sent to the persons subjected.

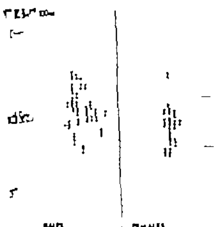


Fig. 1

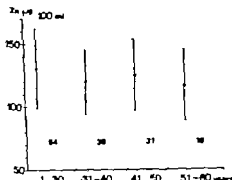


Fig. 2

Serum zinc levels of male and female blood donors from Oslo. Lines indicate mean levels ± S.D

Serum zinc levels in men at different ages. Mean ± S.D

[illegible] men of age 21–40 years were recalled for further examinations because of their serum zinc levels [illegible] were higher than the local mean in 19 men and lower than the mean in 21 men. Only 3 of them [illegible] diagnosed disease. Subject number 24 (Table II) had an arthritis deformans, number 25 [illegible] heart and number 36 (Table III) an arterial hypertension. They used regularly drugs according to their [illegible]. Blood samples of these 40 men were examined using a Technicon Autoanalyzer as [illegible] rough [illegible] of their condition.

[illegible] Blood samples were drawn at 8 [illegible] m. after an overnight fast. Clotted samples were centrifuged [illegible] the serum was used. For hormone determinations serum was conserved at −20 C till the assays were [illegible].

[illegible] The zinc determinations [illegible] ere performed using [illegible] Perkin-Elmer Model 303 atomic [illegible] bsorption [illegible] spectrometer. Standard solutions were prepared using stock solutions (Merck, Germany). The [illegible] due to the differences in [illegible] iscosity between the serum and the aqueous standard solutions was [illegible] voided [illegible] dextran solution instead of water (Hackley *et al.* 1968). Standard solutions contained 154 [illegible] Na and Cl. The samples were undiluted, since optimal sensitivity could be obtained in the recorder [illegible] in that range. Sources of contamination (rubber stoppers etc.) [illegible] ere excluded as far as possible [illegible] haemolysed serum was used.

[illegible] follicle stimulating hormone (FSH) and serum luteinizing hormone (LH) [illegible] are determined by [illegible] double antibody radioimmunoassay (Raeder *et al.* 1973). The second antibody was fixed on [illegible] cellulose according to the method of Wide *et al.* (1969). Kits by CEA-IRE SORIN were used [illegible] LER 907 was used as reference preparation. Radioimmunoassay of serum testosterone was per[illegible] after extraction of 30 µl of serum in diethyl ether (ad 3.0 ml) as described by Dufau *et al.* 1972. [illegible] the statistical treatment of the data regression analysis (Spearman [illegible] correlation coefficient) and [illegible] were used.

Results

[illegible] zinc, sex, age and height. The mean serum zinc level ±S.D of the 154 male blood [illegible] was 123.84 ± 28.49 µg/100 ml and that of the 95 females 111.36 ± 23.40 µg/100 ml. [illegible] ranges [illegible] were 73.5–220 in the men and 63.5–181.5 in the women (Fig. 1). The difference [illegible] between the men and women was highly significant ($p < 0.001$). Fig. 2 shows mean serum [illegible]

TABLE I Serum zinc levels, hormone levels and other data of 15 men aged 28–35 years.

Subject No	Age in years	Height cm	Weight kg	Zinc µg/100 ml	Testosterone ng/ml	FSH ng/ml LER 907	LH ng/ml LER 907	Occupation
1	28	177	87	154	10	63	81	Postman
2	28	—	—	160.5	5.5	163	102	Bus driver
3	33	167	62	131	6.0	170	31	Service station keeper
4	33	173	84	145.5	6.4	97	191	Draftsman
5	33	176	73	220	2.1	170	92	Engineer
6	34	167	74	148	4.2	99	60	Technician
7	35	185	90	127.5	5.[illegible]	170	81	Baker
n, 7								
Mean ±S.D	3[illegible].00 ±2.82	174.16 ±6.82	78.33 ±10.55	155.21 ±30.89	5.6[illegible] ±2.39	125.28 ±43.06	91.14 ±49.80	
8	30	186	84	73.5	16	68	56	Geologist
9	30	—	—	99.5	6.7	135	63	MD
10	30	183	70	10	5.5	156	42	University functionary
11	31	—	—	93.5	6.5	106	63	—
12	3	175	70	101	6.3	149	42	Caretaker
13	34	171	75	93	3.9	9	88	Engineer
14	35	171	81	85	7	117	63	Printer
15	35	—	—	113	13	110	50	Policeman
n, 8								
Mean ±S.D	32.13 ±[illegible].23	177.20 ±6.94	76.00 ±6.36	95.06 ±11.93	8.11 ±4.13	116.12 ±29.45	58.37 ±14.89	

zinc levels (±S.D.) in men at different ages. There was a slight tendency to lower l[evels in] men after the age of 30 years, but the difference was not significant at the 0.05 level (p[...] There were no statistically significant differences in the height between the men w[ith low] serum zinc and the men with high serum zinc (Tables I III).

Serum zinc testosterone and gonadotropins The individual values and mean h[ormone] levels ±S.D for the different groups and other relevant data are given in Tabl[e...] In men aged 36–60 years there was a significant positive correlation between ser[um zinc] and serum testosterone ($p < 0.005$) (Fig. 3) In men of 28–35 years there was no sig[nificant] correlation ($R = -0.43$ $p > 0.10$) between zinc and testosterone No correlation c[ould be] established between serum zinc and serum FSH or LH

Discussion

Earlier literature contains controversial data as to sex difference in serum zinc concen[tration] (Davies *et al* 1968 Halsted *et al* 1970 Lindeman *et al* 1971). According to the [present] results, men have significantly higher serum zinc levels than women although the di[fference] is not great, which would explain the controversy especially in studies with limited [numbers] of cases. The existence of sex difference and the age related decrease in serum zinc, th[e latter] being convincingly proved by Lindeman *et al* suggest that serum zinc and repro[ductive] functions might be in some relation. Also the high concentrations of zinc in the

Tables II and III Serum zinc levels, hormone levels and other data of 25 men aged 36–60 years.

II

Age in years	Height cm	Weight kg	Zinc µg/100 ml	Testosterone ng/ml	FSH ng/ml LER 907	LH ng/ml Ler 907	Occupation
36	175	70	170	6.2	135	77	Agricultural agent
40	172	80	154	16	192	60	Factory worker
43	179	93	157	6.9	92	63	Foreman
44	171	73	171.5	16	78	21	Fisherman
46	176	76	216	8	35	46	Engineer
46	170	95	191	15	142	120	Truck driver
47	168	73	124	4.0	99	21	Truck driver
47	—	—	127.5	5.3	156	53	—
49	182	85	157.5	5.1	142	88	Pensionary
50	173	77	168	7.0	106	16	Pensionary
59	175	89	139	4.7	248	99	Engineer
60	164	90	140	3.0	227	77	Factory worker
47.50 ±7.65	171.36 ±5.14	80.09 ±9.28	159.86 ±27.41	8.10 ±4.76	137.66 ±61.91	61.75 ±32.58	

III

Age in years	Height cm	Weight kg	Zinc µg/100 ml	Testosterone ng/ml	FSH g/ml LER 907	LH g/ml LER 907	Occupation
37	173	75	89.5	6.9	106	26	Forester
37	171	73	99.5	3.7	54	35	Factory worker
38	—	—	99.5	6.5	213	42	Bus driver
39	177	76	103	4.8	270	38	Railroad man
40	—	—	102	6.4	270	113	—
41	180	87	99.5	10	213	116	Truck driver
42	172	78	90.5	3.3	71	53	Inspector
43	172	85	102	4.1	99	35	Truck driver
45	190	92	92.5	6.1	85	38	Agent
49	172	78	86	4.0	170	92	Construction worker
51	178	100	90.5	3.7	120	70	Railroad man
51	179.5	72	95	3.7	135	56	Teacher
56	165	68	84	3.2	128	31	Forester
43.76 ±6.16	175.40 ±6.54	80.36 ±9.60	95.03 ±6.20	5.10 ±1.97	148.76 ±72.42	57.30 ±31.06	

, especially in males, support this possibility (Bertrand *et al.* 1921 Mawson *et al.* 1953).

...viously Coble and his coworkers have found that in severely zinc-deficient Egyptian ...there was a delayed puberty with low plasma testosterone and LH (Coble *et al.* 1971). ...y concluded that zinc deficiency primarily disturbs the pituitary function. However ...he present study serum FSH or serum LH did not show remarkable fluctuations in ...ups with different age or serum zinc levels. Therefore, it is not evident that in nutritionally ...mal adults zinc could interfere with reproductive functions primarily via pituitary

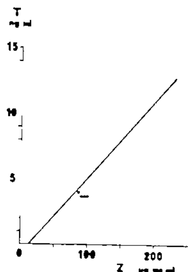


Fig. 3 Correlation between serum zinc and serum testosteron[e in] men aged 36–60 years. Testosterone = 0 06 Zn – 0 5, R = 0 5[5], N[…] $p < 0.005$

Also our recent results (to be published) on zinc-deficient rats show a nearly normal [pitui]tary function in spite of a disturbed testicular function.

At the moment it is not possible to explain why the consequences of low serum zinc ar[e not] uniform. Zinc might be a structural part of proteins involved in the synthesis and tra[nsport] of testosterone It is known to be the active part of several enzymes, the most impo[rtant] metallo-enzymes of zinc being alcohol dehydrogenases and other dehydrogenases (I[…] *et al* 1969) Zinc could also interfere with the hepatic metabolism of testosterone[. It] interferes with hepatic drug metabolism in animals (Becking *et al.* 1970) and prob[ably] in man too (Hartoma *et al* 1977) In fact, in our previous study a prolonged effe[ct of] estrogen was found in zinc-deficient female rats (Hartoma 1975) Altogether there is ev[i]mental evidence that zinc deficiency primarily has an inhibitory effect on the steroid sy[n…] on one side and metabolism on the other Hence, variable testosterone levels wou[ld be] expectable in subjects with differing testicular and hepatic functions.

I wish to thank Professors Väinö Veijola and Jorma Sohlo f[or] their kind permission to use the [atomic] absorption spectrophotometer at the Department of Process Engineering, University of Oulu. [My] thanks are due t[o] Miss Tyyne Määttä and M[r] Valtteri Haverinen for their technical assistance I also [thank] the staff of the Blood Transfusion Service in Oulu for collecting the samples.

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Regional Brain Uptake of Noradrenaline Following Mechanical or Osmotic Opening of the Blood Brain Barrier

By

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Abstract

HARDEBO J. E., L. EDVINSSON E. T. MACKENZIE and CH. OWMAN. *Regional brain uptake of noradrenaline following mechanical or osmotic opening of the blood-brain barrier.* Acta physiol. scand 1977 *101* 342–350

The passage of noradrenaline from the cerebrovascular circulation into the brain (vessel wall and parenchyma) was studied quantitatively and regionally by modification of Oldendorf's technique for determination of brain uptake index. Using ^{14}C-ethanol as the highly diffusible internal standard, the index for noradrenaline varied between 2.7 and 4.5% in different regions, confirming the poor penetration of this neurotransmitter. The barrier was impaired transiently as evidenced by E[illegible] blue extravasation through osmotic opening by internal carotid injection of a hyperosmolar urea solution or mechanical disruption by short lasting elevation of the intracarotid hydrostatic pressure. This resulted in a 3-4[illegible] increase in the passage of noradrenaline from the circulation into the brain.

There is a common opinion based on the limited number of reports so far available, that the blood-brain barrier almost totally prevents the entry of many amines from the blood into the brain substance proper. Additional support for this opinion has come from behavioral and cerebral blood flow studies where little or no effect is obtained following the systemic administration of amines (Purves 1972, Lassen 1974, Edvinsson and MacKenzie 1977). Oldendorf (1971) has demonstrated that both amines and amino acids in fact are extracted from the circulation in the brain but to a highly varying extent. Thus, many of the amines, such as the neurotransmitters, have a "brain uptake index" (obtained during the extraction from the blood) which is only in the range of 2.5 to 4.5 when compared to the uptake of a highly diffusible standard. Essential amino acids, on the other hand, are more readily taken up from the brain circulation, probably with the aid of active uptake mechanisms (Battistin, Grynbaum and Lajtha 1971).

A modified procedure for estimating the brain uptake index was utilized to study regional variations in the uptake of tritiated noradrenaline from the blood to the brain. The ability of the amine to penetrate the blood-brain barrier was also examined under two experimental

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at are known to open the structural barrier by intracarotid infusion of hyper osmotic opening) and by intracarotid injection of blood at high pressure disruption). The impairment of the barrier function and the reversibility of the visualized by administration of Evans blue into the circulation.

Methods

s were carried out on 52 adult male Sprague-Da ley rats, eighing 300–350 g and fed by and tap ater *ad lib* The animals were initially anesthetized in glass jar containing 2 3 racheal cannula as inserted and anesthesia was maintained with mixture of 70 % N_2O The body temperature was maintained around 37°C as measured by rectal thermometer heters were placed in the femoral arteries and veins on both sides One of the femoral arterial used for continuous recording of mean arterial blood pressure and the other artery was blood sampling of blood for measurement of blood gases ($PaCO_2$ and PaO_2) hich, together quantified in 100 µl samples ith MRK II blood gas analyser (Radiometer Copenhagen). were maintained at normocapnia ($PaCO_2$ between 35 and 40 mmHg) The main trunk of nal carotid artery was cannulated centripetally with the catheter opening placed within 1 mm carotid bifurcation, and the remaini g branches of the ipsilateral external carotid artery (In order to facilitate cannulation, the external carotid artery was exposed by dividing the nd into the hyoid bone, hich overlie the distal portion of the artery) Injections through the external carotid artery were then carried into the internal carotid artery directly to the

ing of the blood-brain barrier

s, 1 ml of Krebs-Ringer buffer solution (pH 7 4 37°C) containing 2 M urea[1] was infused on the cannulated external carotid artery to reach the brain. 10 of the animals had received 20 min beforehand (1 ml of 1 5 solution in Krebs-Ringer buffer). This substance binds proteins (Freedman and Johnson 1969) and does not, therefore, penetrate the blood-brain normal conditions. After further 10 min the animals were killed for macroscopic and microscopic examination (see below) In 5 of the animals, the infusion of 2 M urea was min later by bolus containing mixture of radioactive noradrenaline and ethanol (see further 8 animals (used as controls with an intact blood-brain barrier), 1 ml Krebs-Ringer on alone (37°C) as infused during 15 down the external carotid artery followed 10 min radioactive bolus mixture The animals were decapitated 15 later Reversibility of the struc opening was studied in 4 additional animals by administration of Evans blue 30 min after the 2 M urea, Evans blue as allowed to circulate for 20 min before the animal was sacrificed.

Disruption of the blood-brain barrier

s, 1 0 ml of blood (autotransfusion of blood slowly collected from the femoral artery be-fore) as rapidly injected (during 3) into the cannulated external carotid artery to reach the the animals had been given Evans blue 20 min beforehand. The remaining 5 animals received radioactive noradrenaline and ethanol (see below) 10 min after the injection of blood and were

ntration of urea was chosen from pilot study In which the concentration of urea was found west that could open the structural blood-brain barrier as judged by macroscopic extravasa-ns blue (9 of 10 animals, in 3 animals receiving 1 5 M urea no extravasation of Evans blue , hereas in 3 animals marked extravasation was obtained after 3 M urea).
study varying volumes of blood or Krebs-Ringer buffer solution were infused ith different the external carotid artery A rapid injection during approximately 0.2 of 0.2 ml blood or not opened the blood-brain barrier as judged by extravasation of Evans blue macroscopically fluorescence microscope (4 of 5 animals). The same effect as achieved hen 1 ml of blood or tion was infused during 3 (5 of 5 animals) Injection of 0 04 ml Krebs-Ringer buffer solution or 1 ml of buffer solution during 15 s, did not induce any macroscopically detectable extravasation blue (4 4 animals) Consequently the rapid injection of 1 0 ml of blood as selected as the chance to disrupt the barrier mechanically

killed 15 s later. In order to study reversibility of the mechanical barrier disruption, Evans blue was injected 24 h after the autotransfusion of 1 ml blood during 3 s in 4 animals; in order to [illegible] tracheotomy the cannulation of the external carotid artery was performed under brietal-sodium anesthesia in these animals.

Fluorescence microscopy

The brain was removed from animals treated as above as well as from animals perfused with [illegible] through the left ventricle of the heart. Selected regions (frontal, parietal, temporal and occipital cortex with underlying white matter caudate nucleus, thalamus, mesencephalon, and cerebellum) were [illegible] rapidly in a propane-propylene mixture, cooled to the temperature of liquid nitrogen. After subsequent freeze-drying, the specimens were processed for fluorescence microscopy according to the method of F[illegible] and Hillarp involving exposure of the tissue to gaseous formaldehyde during dry conditions (Björklund, Falck and Owman 1972). The Evans blue-albumin complex displays a red fluorescence (Steinwall and Klatzo 1966) under the fluorescence microscopic conditions used.

Brain uptake index

Determination of the brain uptake index was performed by infusion during 1 s of 0.04 ml Ri[illegible]ger buffer solution (37°C), containing a mixture of (−)-noradrenaline-7-^{3}H (New England Nuclear; specific activity 3.0 Ci/mmol concentration 7.5 nmol/ml) and ^{14}C-ethanol (specific activity [illegible] mmol concentration 50 nmol/ml). The bolus mixture was infused through the cannula [illegible] centripetally in the right external carotid artery thus reaching the brain directly as described above. The heart of the animal was stopped with saturated potassium chloride 15 s after administration of the bolus, the animal was instantly decapitated and the head frozen in liquid nitrogen. Various regions (frontal, parietal and occipital cortex with underlying white matter caudate nucleus, thalamus, mesencephalon) from the injected side were chiselled out in a cryostat at −15°C, weighed and placed in liquid scintillation vials containing 0.5 ml Soluene (Packard). When the tissue had been dissolved, [illegible] of Instagel was added. The tissue samples were counted for β-emissions in a Nuclear Chicago [illegible] scintillation counter. Quench correction was performed according to conventional principles.

To calculate the brain uptake index for noradrenaline the method developed by Oldendorf (1971) [illegible] used with two important modifications. Firstly the arterial inflow to the brain was not interfered with by manipulation of the common carotid artery and secondly ^{14}C-ethanol substituted for ^3H_2O as internal standard ("freely" diffusible standard). The ^{3}H and ^{14}C ratio in the tissue is divided by the ratio in the injected mixture and the result multiplied by 100 to provide the uptake of the test substance as percentage of the ethanol uptake, thus

$$\mathrm{BUI} = \frac{\text{tissue} - {}^3\text{H}/\text{tissue} - {}^{14}\text{C}}{\text{mix} - {}^3\text{H}/\text{mix} - {}^{14}\text{C}} \cdot 100$$

Cerebral blood flow

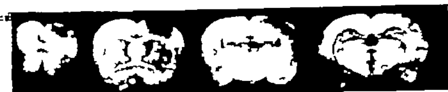
Determination of regional cerebral blood flow (rCBF) was performed by the ^{14}C-ethanol technique according to Eklöf *et al.* (1974). Catheters were placed into the femoral arteries and veins on both sides; the right external carotid was cannulated as described above (for further details, see Edvinsson [illegible] 1977). 10 min after an injection of 2 M urea into the right internal carotid the rCBF was measured [illegible] animals. The mean (±S.E.) flow in regions nourished by the carotid on the injected side was 66±[illegible] 100 g min, compared to 70±8 on the contralateral side. Similarly rCBF was measured in 4 [illegible] 10 min after a bolus injection of blood [illegible] the right internal carotid the mean flow was 72±6 in [illegible] on the injected side, compared to 74±5 on the contralateral side. The slight reductions in flow [illegible] the injected side were not statistically significant.

Statistics

Differences between mean values were assessed by the Student's *t*-test for unpaired data.

Results

All brain regions taken from animals injected with Evans blue, but not treated to [illegible] osmotic opening or mechanical disruption of the blood brain barrier showed no [illegible]scopically visible staining of the parenchyma. Fluorescence microscopy of these co[illegible]



1. Sections of rat brain removed 10 min after injection of M urea bolus into the right internal carotid artery. Patchy areas of extravasated Evans blue-albumin complex are seen in regions supplied by internal carotid artery

nals revealed greenish background fluorescence in the brain tissue The well-known appearance and distribution of the formaldehyde-induced fluorescence in the monoamine neuron systems were recognized. The lumen of vessels everywhere in the brain contained Evans blue (in animals not perfused at sacrifice), which could easily be visualized by its bright red fluorescence. No Evans blue could be found in the vascular walls except for a fluorescence restricted to the intima of the pial vessels. After intracarotid injection of 2 M urea to induce osmotic opening of the barrier or an intracarotid injection of blood or buffer solution (1 ml during 3 s) to induce mechanical disruption of the barrier a blue staining with varying localization in individual animals was seen macroscopically in superficial and deep structures of the hemisphere on the injected side, whereas the brain stem including cerebellum remained unstained (Fig. 1). These signs of extravasated Evans blue correspond to the regions reached by the initial high concentration of urea or by the initial high intravascular pressure induced by the rapid injection of blood or buffer solution. In the fluorescence microscope, a red fluorescence of patchy distribution was seen in all parts of the parenchyma of those regions which showed a macroscopically visible staining. In animals with mechanically induced disruption the leakage was noticed particularly at the level of small arterioles, capillaries and veins. The green formaldehyde-induced neuronal fluorescence in these regions of extravasation was not as well developed as in the remainder of the parenchyma sometimes neurons were even seen to be loaded with red-fluorescent material (*cf* Flodmark *et al.* 19). Occasionally a red fluorescence was also seen in the wall of small vessels, particularly capillaries, in the parenchyma outside areas showing extravasation. For obvious reasons, this staining of the vessel wall was best seen in animals perfused at sacrifice. In animals with no macroscopical extravasation of Evans blue, no sign of barrier damage—in the brain regions studied—appeared in the fluorescence microscope either Reversibility of the damage was indicated by absence of extravasation of Evans blue (macroscopically and in the fluorescence microscope) in animals receiving the dye 30 min after 2 M urea, or 24 h after rapid injection of blood.

The brain uptake index for noradrenaline in animals with an intact blood-brain barrier varied according to the region studied, as shown in Table I It was slightly higher in the cortical tissue than in the deeper regions of the hemisphere. After intracarotid injection of urea the brain uptake index for noradrenaline was markedly increased—by as much as 4 times—in those brain regions straightly reached by the essentially undiluted urea bolus (parietal, occipital and frontal cortex preparations, caudate nucleus and thalamus). The mean uptake in these brain regions was enhanced from 3 6% to 13.8% (Fig. 2). Accordingly

killed 15 s later. In order to study reversibility of the mechanical barrier disruption, Evans bl injected 4 h after the autotransfusion of 1 ml blood during 3 s in 4 animals; in order to tracheotomy the cannulation of the external carotid artery was performed under brietal-sodium anesthesia in these animals.

Fluorescence microscopy

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To calculate the brain uptake index for noradrenaline the method developed by Oldendorf (1971) was used with two important modifications. Firstly, the arterial inflow to the brain was not interfered with by manipulation of the common carotid artery and secondly, ¹⁴C-ethanol substituted for ³H₂O as the internal standard ("freely" diffusible standard). The ³H and ¹⁴C ratio in the tissue is divided by the same ratio in the injected mixture and the result multiplied by 100 to provide the uptake of the test substance as a percentage of the ethanol uptake, thus

$$\mathrm{BUI} = \frac{\text{tissue } {}^{3}\mathrm{H}/\text{tissue } {}^{14}\mathrm{C}}{\text{mix } {}^{3}\mathrm{H}/\text{mix } {}^{14}\mathrm{C}} \times 100$$

Cerebral blood flow

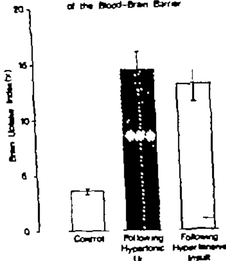
Determination of regional cerebral blood flow (rCBF) was performed by the ¹⁴C-ethanol technique according to Eklöf *et al.* (1974). Catheters were placed into the femoral arteries and veins on both sides, and the right external carotid was cannulated as described above (for further details, see Edvinsson et al. 1977). 10 min after an injection of 2 M urea into the right internal carotid the rCBF was measured in [illegible] animals. The mean (±S.E.) flow in regions nourished by the carotid on the injected side was [illegible] 100 g min, compared to 70±8 on the contralateral side. Similarly rCBF was measured in 4 rats 10 min after bolus injection of blood into the right internal carotid, the mean flow was 77±6 [illegible] on the injected side, compared to 74±5 on the contralateral side. The slight reductions in flow on the injected side were not statistically significant.

Statistics

Differences between mean values were assessed by the Student's *t*-test for unpaired data.

Results

All brain regions taken from animals injected with Evans blue, but not treated to induce osmotic opening or mechanical disruption of the blood brain barrier showed no macroscopically visible staining of the parenchyma. Fluorescence microscopy of these cor[illegible]



3. Mean values of brain uptake index for nor... regions supplied by the internal carotid (cf. Table I). Untreated controls and animals ...ed to osmotic opening of the blood-brain ... by 2 M urea or mechanical disruption of the ... by ... pulse of hydrostatic pressure. The dif... between the mean values is highly significant ($P < 0.001$)

...r osmotically from the cerebral endothelial cells resulting in their shrinkage, which is ...sed to transiently open up the barrier at the tight junctions between contiguous endo...al cells (Rapoport *et al.* 1972). The local concentration of urea must be high to have ... effect (Rapoport *et al.* 1972), in accordance with the present observation that no macro...ically or microscopically visible extravasation of Evans blue occurred in regions outside ...upply of the internal carotid artery (into which the urea bolus was injected), even ...gh these regions were exposed to the urea diluted in the systemic circulation. The thresh... concentration of the urea solution needed to induce barrier opening was in the magni...e of 2 M, which is in agreement with observations in the rabbit (Rapoport *et al.* 1972). ...as shown that the barrier opening was reversible within 30 min, confirming earlier ...ings by Rapoport *et al.* (1972).

...pulse of hydrostatic pressure, elevating the carotid artery pressure above 200 mmHg, ...been shown to open the blood-brain barrier in the rat as evidenced by extravasation of ...ns blue (Rapoport and Thompson 1975). This barrier damage was confirmed in the ...ent series of expts. and the extravasation was predominantly noticed at the level of the ...ll vessels. Acutely induced extreme hypertension also causes blood-brain barrier ...age, preferentially in small arterioles and capillaries, probably related to pressure-...ed overdistension of the vessels (Johansson, Strandgaard and Lassen 1974, MacKenzie ...al. 1976 c). It may be assumed that the same mechanism is responsible for the mechanical ...ier disruption induced by a pulse of hydrostatic pressure. At the ultrastructural level, ...barrier damage in acute hypertension comprises formation of channels in the cytoplasm ...ndothelial cells, an increased transendothelial pinocytosis and, rarely opening of the ...ht junctions (Hansson, Johansson and Blomstrand 1975). It is probable, but not yet shown, ...at the hydrostatic pressure-induced barrier damage has a similar ultrastructural appear...ce. Reversibility of barrier disruption was shown to occur within 24 h, the time interval ...osen on the basis of similar expts. by Johansson (1974).

TABLE I Values for brain uptake of H-noradrenaline before and after osmotic opening or mechanical disruption of the blood-brain barrier by injection of 2 M urea or a rapid bolus of blood respectively into the internal carotid artery Mean values ± S.E. of 6 animals in each group (for mesencephalon[a] only 3 values were obtained).

Brain region	Intact barrier	After Osmotic opening	After mechanical disruption
Parietal cortex	4.26 ± 0.44	16.61 ± 3.00	15 61 ± 3.86
Occipital cortex	4 46 ± 0 45	17.53 ± 3.59	14 09 ± 2.93
Frontal cortex	3 40 ± 0.29	11 40 ± 1.97	9 78 ± 1 66
Caudate nucleus	2.83 ± 0.38	12.48 ± 3.04	11.35 ± [illegible]
Thalamus	2.69 ± 0 40	10 93 ± 2.62	11.26 ± 3 0[illegible]
Mesencephalon	3.98 ± 0 46	3 93 ± 0 49 n s.	2.93 ± 0 06 n s.

Comparison of mean values according to Student's t-test 0 001 < p < 0 01 *0 01 < p 0.05, n.s. non-significant.
[a] That radioactivity sufficiently above back-ground reached mesencephalon at all, although this region is not directly supplied by the internal carotid artery may be explained by a transient change in the direction of flow during injection.

the index was not affected in mesencephalon a region supplied by the vertebro-basilar system (Table I) Similarly after mechanical disruption of the barrier the brain uptake index was enhanced over 3-fold in those parts of the brain affected by the high intraluminal pressure induced by the rapid injection of blood (the mean uptake increased from 3 6% to 12.4% Fig. 2) As could be expected, the uptake was not enhanced in mesencephalon (Table I)

Discussion

The ability of many amines to penetrate from the circulation into the brain parenchyma is generally considered to be minimal, if any Fluorescence microscopy after systemic administration of neurotransmitter amines, such as noradrenaline has indicated that the passage is predominantly impeded already at the luminal surface of all pial and parenchymal vessels but in addition at the level of endothelial cells and pericytes of cerebral capillaries and veins (Hardebo *et al* 1977) There is evidence that monoamine oxidase, present in the vessel wall is involved in this barrier mechanism so that the monoamines are trapped by an enzymatic breakdown process (Bertler *et al* 1966 MacKenzie *et al* 1976 b, Hardebo *et al* 1977). There is no reason to believe that the monoamine barrier is directly associated with the particular ultrastructural organization of the cerebrovascular endothelium. Thus, for example, electron microscopy studies have not demonstrated any differences between the endothelial cells and cellular interconnections on the arterial side on one hand and capillaries and veins on the other (Reese and Karnovsky 1967), although the passage of amines is different in these two parts of the vascular bed, as shown by fluorescence microscopy (Hardebo *et al* 1977).

It has recently been shown that intravascular administration of hypertonic solutions e.g urea allows large molecules that do not normally penetrate the blood-brain barrier (like horseradish peroxidase and Evans blue-albumin complex) to pass from the blood to the brain tissue (Rapoport, Hori and Klatzo 1972, Brightman *et al* 1973). This was confirmed in the present study using Evans blue. It has been suggested that urea extracts intracellular

...It has been suggested (Edvinsson *et al.* 1972) that the low penetrability of noradrenaline ...the intact blood-brain barrier may account for the relatively weak effect of the amine ... cerebral blood flow when injected into the circulation. This assumption has found direct ...port in expts. showing that osmotic opening of the barrier markedly alters the nor...renaline-induced effect on local cerebral blood flow (MacKenzie *et al.* 1976 a, Edvinsson ...l. 1977)

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A useful method for quantitative measurement of the passage of a wide variety of solutes from blood to brain vessel walls and brain parenchyma has been developed by Oldendorf (1971). According to this method, the substance to be tested is injected in its radioactive form into the common carotid artery together with a radiolabelled highly diffusible compound used as internal standard, and the radioactivity is measured in the brain after a single capillary passage. Recently this method has been slightly altered by Oldendorf and Braun (1976). The original method was modified in the present study by employing ^{14}C-labelled ethanol instead of tritiated water as the internal standard since it has been demonstrated that the former diffuses significantly better into the brain (Raichle *et al.* 1976). Using tritiated water as standard Oldendorf found a mean brain uptake index of 4.5% for noradrenaline, whereas in the present expts., using ^{14}C-ethanol as standard, a value of 3.6% was obtained. This difference can be explained on the basis of the data given by Raichle *et al.* (1976), who found that only 93% of an injected bolus of labelled water is freely exchangeable with brain provided the cerebral blood flow is about 50 ml/100 g min; the comparable figure for ethanol is 97%. At higher flow levels the extraction tends to be reduced. Catheterization of the external carotid artery on one hand and the injection of urea (MacKenzie *et al.* 1976) or a bolus of blood (see Methods) on the other did not cause any significant change in the cerebral blood flow at the point of time when the brain uptake index was estimated. Further, it is highly unlikely that the trace amounts of noradrenaline that are injected can itself affect the CBF. This would mean that with the flows obtained in the present study the absolute values for the brain uptake index will, if anything, be slightly underestimated. Since the use of ethanol as standard would, on the other hand, tend to slightly overestimate the true uptake of amines, the technique allows for a good approximation of the penetration of various test solutes. The brain uptake index for noradrenaline was found to vary between 2.7 and 4.5% depending on the region studied. A second modification of the Oldendorf technique comprised injection into the internal carotid artery via the cannulated external carotid and not through a needle in the common carotid artery. This procedure obviates any interruption of blood flow through the internal carotid artery and also lowers the risk of vasospasm of this artery frequently occurring when the common carotid artery is manipulated.

The brain uptake index was utilized not only to determine the normal regional passage of noradrenaline into the brain, but also to quantify the impairment of barrier function induced by osmotic opening or mechanical disruption. It was found that in those regions of the brain reached by high concentrations of urea, as evidenced by extravasation of Evans blue (reflecting areas where the barrier had been disrupted mechanically), the brain uptake for noradrenaline was enhanced more than 3-fold. In both series of expts., the relative increase in brain uptake was similar in all brain regions studied, indicating that the extent of barrier damage was of the same magnitude in these regions. Since the standard used, ethanol, is not freely permeable, as mentioned above, an opening of the barrier may tend to enhance the passage also of the standard and possibly influence the brain uptake index. However, any increase in the passage of ethanol can only be minimal, resulting in but a very slight underestimation of the index, and it may even be proportional to the increase in the passage of the test substance, and hence still give a correct estimation of the index.

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Low Intensity Training, Inactivity and Resumed Training in Sedentary Men

By

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Abstract

Örlander, J., K.-H. Kiessling, J. Karlsson and B. Ekblom. *Low-intensity training, inactivity and resumed training in sedentary men.* Acta physiol. scand. 1977. *101*. 351–362.

Effects of a low intensity training regimen, consisting of two 7-week periods with an interspersed week inactivity period were investigated in 16 sedentary men. A follow-up was made on 7 subjects after additional weeks training. Systemic as well as local effects were studied using exercise tests and leg muscle biopsies. The two 7-week training periods both resulted in a 6 % increase in $\dot{V}o_2$ max and a lowered heart rate during submaximal work. No persisting training effects were detected by exercise tests after inactivity. In skeletal muscle, however, striking differences in enzyme activity pattern and ultrastructure were observed between the two periods, indicating that some training effect of importance for muscle metabolic potential might have persisted during inactivity. It is suggested that such an effect might be associated with the local oxygen supply. During the 38-week training period there was a large increase in muscle metabolic capacity but no change in maximal oxygen uptake. This separation of systemic and local training effects indicates a lack of a direct causal relationship between muscle metabolic potential and maximal oxygen uptake. It is suggested that the elevated muscle oxidative capacity is of importance for an increased endurance capacity.

Key words: Exercise, training, inactivity, oxygen uptake, muscle metabolism, muscle ultrastructure

It is well established that regularly performed endurance-type training will induce significant adaptations at the systemic as well as at the muscle cell level. Improvements in cardiovascular function (e.g. Saltin *et al.* 1968, Ekblom 1969) are accompanied by an increased metabolic capacity in skeletal muscle, resulting in augmented working capacity and endurance (e.g. Holloszy and Booth 1976).

The circulatory effects of low intensity training have been evaluated in many studies—e.g. Kilbom (1971) (for other references, see Pollock 1973). However, little attention has hitherto been paid to the effects on local muscle physiological factors of the kind of low-intensity training that can be carried out mainly unsupervised by previously sedentary people. The present investigation has therefore been concentrated on the following three aims: (1) to study the effects of training of low intensity on sedentary people, (2) to establish

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Effects of low intensity training regimen, consisting of two 7-week periods with an interspersed inactivity period were investigated in 16 sedentary men. A follow-up was made on 7 subjects after additional weeks training. Systemic as well as local effects were studied using exercise tests and leg muscle biopsies. The two 7-week training periods both resulted in 6 % increase in $\dot{V}o_2$ max and lowered heart rate during submaximal work. No persisting training effects were detected by exercise tests after inactivity. In skeletal muscle, however, striking differences in enzyme activity pattern and ultrastructure were observed between the two periods, indicating that some training effect of importance for muscle metabolic processes might have persisted during inactivity. It is suggested that such an effect might be associated with the local oxygen supply. During the 38-week training period there was a large increase in muscle oxidative capacity but no change in maximal oxygen uptake. This separation of systemic and local training effects indicates lack of a direct causal relationship between muscle metabolic potential and maximal oxygen uptake. It is suggested that the elevated muscle oxidative capacity is of importance for an increased endurance capacity.

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It is well established that regularly performed endurance-type training will induce significant adaptations at the systemic as well as at the muscle cell level. Improvements in cardiovascular function (e.g. Saltin *et al.* 1968, Ekblom 1969) are accompanied by an increased metabolic capacity in skeletal muscle, resulting in augmented working capacity and endurance (e.g. Holloszy and Booth 1976).

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to what extent training effects may persist during a period of inactivity and possibly affe the results of renewed training, and (3) to investigate the additional effects of prolong (9 months) self-governed training. Conventional submaximal and maximal exercise te together with muscle biopsy have been applied to study cardiovascular functions as well skeletal muscle energy metabolism and ultrastructure

A preliminary report concerning a part of this work has been given elsewhere (Örland and Kiessling 1976)

Material and Methods

Subjects 24 healthy sedentary men volunteered to participate in the investigation. They were all whi colla workers, and had n t been engaged in any regular training for several years, if at all. Eight subjec discontiued training, 2 for long periods of sickness (colds) and 6 due to reasons related to their jobs. Of the remaining sixteen were included in the results. Pertinent anthropometrical data for these were 35 ± 1 years (25–43), height 181 ± 2 cm (173–194) and weight 77.0 ± 3.2 kg (64 0–108.7) (mean±SE range withi brackets) Eight of them were smokers, none stopped smoking during the training

Training regimen. The training programme consisted of two 7-week periods of low intensity train (periods I and II, respectively) with an 8-week inactivity (return to pre-training activity level) period between. On the average, each subject completed three training sessions per week running or walking km, swimmi g and making callisthenics. The time for each training session was 20–30 min and the intens for each type f exercise was prescribed to tax the oxygen transport system not above 80% of maximu This was frequently checked by measurements f the heart rate (direct or by tape recorder) After the seco period most of the subjects co tinued this type of training on thei own. Seven of the original 16 subje were re-examined 38 weeks after completion of the main study All but one of these took part in every training programme during part of that time (which will be referred to as period III).

Exercise test Before this investigation started, preliminary tests were carried out in order to familia the subjects with the test methods used and t obtain preliminary alues on V_{O_2} ma and maximal w loads to use.

At each of the 5 test occasions the same experimental procedure was used. One 6 min submaximal w load (averaging 750 and 1 050 kpm min t the first and second training period, respectively) as p f rmed on mechanically braked bicycle ergometer (Monark) and one exhaustive run (ork time 4-6 m was performed on a moto driven treadmill On each work load, oxygen uptake (V_{O_2}), heart rate (HR blood lactate ([HLa]), hemoglobin ([Hb]) hematocrit (Hct) and subjective rate of perceived exert (RPE) was determined. The speed and slope during the maximal run were individually chosen to keep t work time within 4 to 6 min.

V_{O_2} was determined with the Douglas bag method and the expired air analyzed with the Haldane tech nique. HR was calculated from the ECG recorded each minut during submaximal exercise and co tinuously t the end of the maximal run [Hb], Hct nd [HLa] (Barker and Summerson 1971) ere dete mined in blood samples, taken from a prewarmed fi gertip RPE was determined according to the metho described by Borg (1962).

Muscle biopsies Bef re and after each training period, biopsies were taken from the musculus vas *lateralis* with the needle technique (Bergström 196_), alternating between the left (first tw biopsies) and the right (last three biopsies) leg The muscle specimens were divided into 3 portions one for the assay enzyme ctivities, one for histochemistry and one for electron microscopy

Enzyme assays. The activities of 6 enzymes, each representing a subsystem i energy metabolism, w measured. The enzymes and subsystems were· Phosphofructokinase PFK (E.C._.7 1 11)—*glycolys* lactate dehydrogenase, LDH (E.C.1 1 1.27)—*lactat formation*, 3-hydroxyacyl-CoA dehydrogenase, HAD (E.C.1 1 1.35)—*fatty acid β-oxidation*, citrate synthase, CS (E.C.4 1.3 7)—*citric acid cycle* cytochrom oxidase, cytox (E C.1.9 3 1)—*respiratory chain*, nd mitochondrial α-glycerophosphate dehydrogenase m-GPD (E.C.1 1.99.5)—*glycerophosphate shuttle*

The biopsy specimens (15–50 mg) were homogenized with 19 times the amount (w/) of ice-cold pot sium bicarbonate, 62 mM, pH 7 4 containing 0.15 M KCl nd 6 mM EDTA in small ice-cooled, all gla Potter Elvehjem homogenizer The homogenization was performed by hand in 6 periods of 30 s ith intervals to avoid rises in temperature. The resulting crude homogenat was kept ice-cold and as s ... diluted prior to activity determinations

FK activity was estimated according to Shonk and Boxer (1964), LDH and HAD by the methods of ... *et al.* (1969), CS as described by Srere (1969), and cytox according to Wharton *et al.* (1969). α-GPD ... was estimated by two different methods: the first two biopsies were analyzed polarographically ... Clark oxygen electrode and ... reaction mixture containing 50 mM potassium phosphate, pH 7.5, ... cytochrome c, 4.5 mM α-glycerophosphate, 1 μM rotenone and 100 μl homogenate in ... final volume ... The following two biopsies were assayed according to Bass *et al.* (1969) as modified by Pette *et al.* ... α-GPD activity was not determined in the fifth biopsy. The spectrophotometrical assays were run ... 3 C in ... Beckman model 25 recording double beam spectrophotometer and polarographical assays made at 38 C.

... was estimated according to Lowry *et al.* (1951).

... enzymes, co-enzymes and enzymes used in the assays were purchased from Sigma Chemical Co. or ... Boehringer Mannheim GmbH.

... histochemistry. The muscle specimen was frozen in isopentane cooled by liquid nitrogen. Transverse ... sections (10 μm) were cut in ... cryostat at −20°C, mounted on cover glasses, and stained for myofibrillar ... ATPase (Padykula and Herman 1955) after preincubation at pH 10.3 (for details, see Gollnick *et al.* 1972). ... electron microscopy. Small pieces of muscle tissue were fixed in ice-cold 2% osmium tetroxide in veronal ... buffer, pH 7.4, for two hours, ... washed, dehydrated in ethanol and propylene oxide, and embedded in Epon. ... Longitudinal sections, approximately 70–80 nm thick, were cut in ... LKB Ultrotome I, stained with uranyl ... acetate and lead citrate and examined in ... Siemens Elmiskop 101 or Hitachi HU 11E 1 electron microscope.

... 17 micrographs (final magnification ... about 24 000) were taken at random from 2–4 muscle fibres per ... and analyzed stereologically according to Weibel (1969). To estimate the number of mitochondria ... unit volume (N_V), the formula $N_V = K/\beta \sqrt{N_A^3/V}$ (Weibel 1969) was applied. The size distribution ... factor K was taken to be 1.07 and the shape coefficient β to be 1.6 (which refers to spheroids with an ... axial ratio of 2). Mitochondrial mean volume was calculated by dividing the volume fraction V by the ... This technique gives only rough estimates of the true mitochondrial number and mean volume, since ... skeletal muscle mitochondria often are irregularly shaped. For comparative purposes, however, the method ... is applicable.

... Recently Eisenberg and Kuda (1976) have shown that the three fibre types of guinea pig skeletal muscle ... can be identified in electron micrographs with 90 per cent success rate on the basis of Z-line width and ... mitochondrial density. Unfortunately we found neither bimodal Z-line width distribution nor relationship between Z line width and mitochondrial volume when applying the same technique to 138 micrographs from our 16 subjects. This may be due to less clear-cut differences between the fibre types in human muscle. The fine structure of the M-line is another parameter that has been used to discriminate human fibre types (Sjöström and Squire 1977). Such an analysis was prohibited by the insufficient resolution obtainable ... our strongly contracted needle biopsy specimens. Therefore, no unambiguous identification of ... types could be made.

It was however observed that, although the Z-line width was unimodally distributed within the total ... volume, it was possible to discern two groups of Z-line widths in almost all individuals. By designating ... fibres with broad Z-lines as slow twitch (ST) fibres (*e.g.* Wroblewski and Jansson 1975), ... proportion of ... fibres was obtained for the whole subject group, that agreed within 2 per cent with the histochemically ... obtained mean value. Thus, the morphometrical data can be regarded as representative for the whole ... even though the individual values may be affected by the low number of fibres analyzed.

... Statistics. Conventional statistical methods were applied. Student's *t*-test was used to test differences ... between means, p < 0.05 being considered significant. Correlations were shown by linear regression analysis.

Results

Exercise tests. The results from the exercise tests are shown in Table I.

The group mean $\dot{V}_{O_2}$ max was the same before the training started in period I and in period II. The group mean increase was also about the same in the two training periods (6.1 and 5.9%, respectively, both p < 0.05). The HR at the submaximal work load ... lower after each training period (both p < 0.05). The increase in oxygen pulse ($\dot{V}_{O_2}$/HR) during the submaximal work was from 13.8 to 14.5 ml O_2 beat^{-1} (5.1%, p < 0.05) in period

TABLE I Body weight oxygen uptake ($\dot{V}_{O_2}$), heart rate (HR), blood lactate ([HLa]) and rate of perce[illegible] (RPE) during submaximal and maximal exercise before and after each training or [illegible] Note that the submaximal load was different during training periods I and II (see Material and [illegible] Values are means ± S E. denotes significant difference (p 0.05) when compared with the v[illegible] the respective training period.

Test occasion	n	Body weight kg	Submaximal exercise $\dot{V}_{O_2}$ l × min	HR beats × ml	[HLa] mM	RPE, points "Local"	"Central"	Maximal exercise $\dot{V}_{O_2}$ l min	ml min⁻ kg⁻¹	HR beats min⁻¹	[HLa] mM	[illegible]
Before training period I	16	77.0 ±3.2	1.88 ±0 0	136.9 ±3.2	3.3 ±0.3	11.8 ±0 4	1 0 ±0.3	3.37 ±0 11	44 ±1.2	193 4 ±1 9	91 ±8 5	[illegible]
After training period I	16	77 3 ±3 3	1 85 ±0 02	1 7.2 ±3 4	2 7 ±0.3	11 2 ±0.5	11 1 ±0.5	3 60* ±0 12	47 1 ±1.3	189 6 ±.9	11 7 ±0 6	[illegible]
After inactivity	16	76 1 ±3.0	.44 ±0 13	156 6 ±4 8	7.0 ±0 8	14.6 ±0.6	13 3 ±0 4	3.38 ±0 1	44 7 ±1.3	190 9 ±.9	10 8 ±0 6	[illegible]
After training period II	16	75 9 ±2.9	.44 ±0 13	146.3 ±4.	5 7 ±0 6	12.9 ±0 7	12 0 ±0.5	3 58 ±0.14	47.5 ±1.2	186 8 ±2 6	11 3 ±0 4	[illegible]
Before training period III	7	80. ±5 7	—	—	—	—	—	3 91 ±0 13	49 9 ±2.1	191 4 ±3 1	11 5 ±0 4	[illegible]
After training period III	7	81.8 ±4 9	—	—	—	—	—	3.84 ±0 08	47 6 ±2.7	191 3 ±3.3	11 8 ±0 7	[illegible]

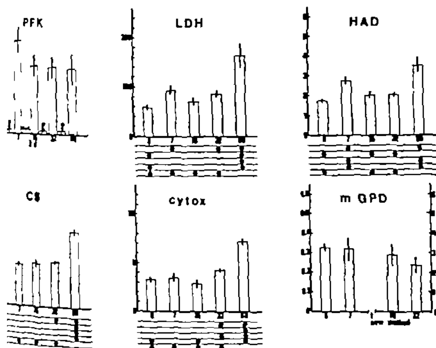
I and from 15 5 to 16.6 ml O beat (7 1 %, p 0 05) in period II During both pe[illegible] there was a tendency (p 0 05) towards a reduced [HLa] and decreased "local and cen[illegible] RPE scores at submaximal exercise During maximal exercise HR [HLa] and RPE s[illegible] were unchanged

In the follow up tests after period III there was a slight drop in $\dot{V}_{O_2}$ max in the 7 [illegible]jects from 3 91 l min to 3 84 l min (p 0 05) Since the mean body weight incr[illegible] somewhat during the period (from 80 2 to 81 8 kg) $\dot{V}_{O_2}$ max per kg body weight decr[illegible] from 49.9 to 47 6 ml min kg (6 2%, p 0 05) HR [HLa] and RPE scores d[illegible] maximal exercise were unchanged

[Hb] and Hct values at rest and during exercise were not affected by the training reg[illegible]

Fibre composition The percentage of slow twitch oxidative fibres (%ST) in the [illegible] *lateralis* was unaffected by the training regimen, viz 39 7 ± 3 7 (15), 35 8 + 4 0 (12), 36 0 [illegible] (14), 39.9 ± 2.2 (15) and 39 4 ± 5.2 (7) (means ± S E. number of subjects within brac[illegible] before and after period I after inactivity after period II and after period III respect[illegible] Some biopsies could not be analyzed for technical reasons No attempts to subdivide [illegible] twitch fibres into A, B and C groups (Brooke and Kaiser 1970) were made

The data allowed an assessment of the degree of variability in fibre composition be[illegible] biopsy specimens taken at different times and sites from the same muscle. The mea[illegible] ference between biopsies was 7.5 ± 6.0 (S D) %ST (48 pairs) wh ch was almost ide[illegible]



Enzyme activities in skeletal muscle before and after each training or inactivity period. Columns mean ± S.E. for 16 subjects (7 at 60 weeks). The activities are given as µmol min⁻¹ (g wet wt)⁻¹ phosphofructokinase (PFK), lactate dehydrogenase (LDH), 3-hydroxyacyl-CoA dehydrogenase (HAD) citrate synthase (CS), and as µmol O_2 min⁻¹ (g wet wt)⁻¹ for cytochrome oxidase (cytox). For mitochondrial glycerophosphate dehydrogenase (m-GPD), the first two values are given as µmol O_2 min⁻¹ wet wt)⁻¹ and the last two as µmol min⁻¹ (g wet wt)⁻¹ (for details, see Material and Methods section). Significant differences (p < 0.05) are indicated by asterisks below each diagram.

the corresponding value when biopsies taken from alternate legs in the same subject compared (7.4 ± 6.0 %ST 73 pairs). This variability was slightly greater than the one reported by Gollnick et al. (1972), in fact possibly due to differences in the size of the analyzed muscle samples. Since the mean %ST was 38.0 in each of the legs (all biopsies included), the data indicate that the two vastus lateralis muscles in the same individual may be regarded as identical as far as fibre composition is concerned.

During period I there was a correlation between the %ST and the increase in V_{O_2} max (0.64, p < 0.05). No such correlation was present during periods II and III or when the various periods were treated together.

Enzyme metabolism. The effects of the various periods of training and inactivity on the activities of the 6 selected enzymes are shown in Fig. 1.

The first training period resulted in significant increases in the activities of LDH and HAD indicating anaerobic capacity and fatty acid β-oxidation, respectively. HAD dropped significantly during the inactivity period, and was then unaffected by the second training period. During the latter period mitochondrial oxygen consumption capacity represented by cytox, increased by 50 per cent. Furthermore, the activities of LDH and of the citric acid cycle enzyme CS were elevated as compared to before the first training period. The 3# sub-

sequent weeks of self-governed training (period III) produced large increases in LDH, HA CS and cytox activities, indicating further augmentations both in anaerobic capacity and the capacity to oxidize fatty acids. The glycolytic pathway represented by PFK, was affected by the training regimen. m-GPD representing the glycerophosphate shuttle, show no effects of training.

Pette and coworkers (Bass *et al* 1969 Staudte and Pette 1971) have demonstrated relations between the activities of enzymes from different metabolic pathways, suggesting pattern of functional coordination. One of the most prominent correlations is the one between CS and HAD In the present material, this correlation was found before (r $p < 0.01$) and after ($r = 0.81$ $p < 0.001$) the first training period, and at the 60 week occasion ($r = 0.90$ $p < 0.001$) No significant correlation was, however present bef ($r = 0.33$) or after ($r = 0.39$) the second training period, suggesting a loss of coordination.

Studies on mixed human muscles with differing fibre composition, and on individ muscle fibres (Essén *et al* 1975 Sjödin 1976, Thorstensson 1976) have shown that twitch and fast twitch fibres differ in their metabolic profiles. The present data failed reveal any relationships between fibre composition and enzyme activities, with the exc tion of LDH versus %ST after period I ($r = -0.62$, $p < 0.05$) and m-GPD versus %ST before period II ($r = -0.70$, $p < 0.01$).

The muscle protein content changed in response to training and inactivity and was 11 0.5 (16), 13.4 ± 0.9 (16) 11.6 ± 0.6 (16), 14.1 ± 0.7 (16) and 9.3 ± 0.6 (7) (per cent of weight, means ± S E. number of subjects within brackets) at the start of the trial, period I after inactivity after period II and after period III respectively The prot content was significantly higher after period II than before periods I and II The last week) value was significantly lower than all the other values. This was not due to a l protein content in the 7 subjects before period III (mean value 13.7%).

Muscle ultrastructure Data on volume fraction, number per unit volume or sarcom and mean volume of mitochondria, as well as the volume fraction of lipid droplets bef and after each training or inactivity period are given in Fig. 2.

The only observed ultrastructural effect of the first training period was a doubling of t intracellular triglyceride content This was, however restored to pretraining levels after inactivity period and no corresponding increase was observed during the subsequent train periods. During the second training period, the mitochondrial density in the fibrillar sp tended to increase ($p < 0.1$) and at 60 weeks it was significantly greater than before train This increase seemed to be due mainly to a rise in mitochondrial number indicated by tendency towards an increased mitochondria-to-sarcomeres ratio (N_s, Fig. 2, lower left).

Essentially the data showed no clear-cut effects of the training regimen on subsarcolem mal mitochondria This may be a true phenomenon but no definite conclusions can be dra because of the relatively sparse occurrence of subsarcolemma zones in the micrograp resulting in a small sample.

A correlation between mitochondrial density in the fibrillar space and cytox activity present after the first ($r = 0.53$ $p < 0.05$) and second ($r = 0.64$ $p < 0.05$) training periods

Smokers vs non-smokers. Before training period I smokers ($n = 8$) had significant lower cytox activities (2.60 ± 0.26 *vs* 4.02 ± 0.32 μmol O min wt) means ± S

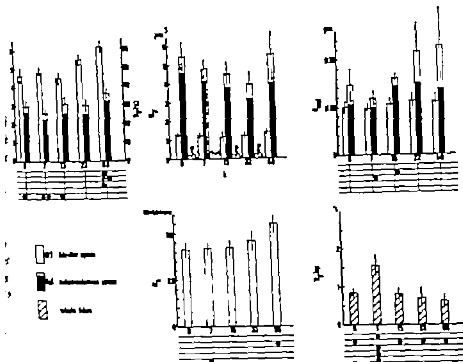


Fig. 2. Volume fraction (V_V), number per unit volume (N_V) and mean volume (V_{mit}) of mitochondria in fibrillar and subsarcolemmal spaces of skeletal muscle fibres, number of mitochondria per sarcomere (N_s) and volume fraction of lipid droplets (V_V-lip) before and after each training or inactivity period. Columns denote means ± S.E. for 16 (0 weeks), 15 (7 weeks), 10 (15 weeks), 10 (22 weeks) and 7 (60 weeks) subjects. Significant differences ($p < 0.05$) are indicated by asterisks below each diagram. Note that numbers and mean volumes of mitochondria are estimates of the true values (see *Material and Methods section*).

and fibrillar space mitochondrial densities (3.90 ± 0.34 vs. 5.45 ± 0.47 %, means ± S.E.) than non-smokers (n = 8). For cytox, this difference persisted during period I, and then disappeared. No other significant differences between smokers and non-smokers in any studied parameter were observed at any time during the investigation.

Discussion

Oxygen transport system. In the present investigation, the "classical" effects of a physical training programme on circulation were demonstrated. Thus, in parallel with the improved physical performance capacity as judged from work time and work load during the maximal exercise tests, the maximal oxygen uptake increased and the heart rate and rate of perceived exertion during the submaximal work load decreased after as compared to before each training period in all subjects. Furthermore, during the physical inactivity between the first two training periods these values returned to pre-training levels.

The increase in $\dot{V}_{O_2}$ max was almost identical during the two training periods (Table I). However there was no intraindividual correlation between the increases in maximal aerobic

sequent weeks of self-governed training (period III) produced large increases in LDH, HAD, CS and cytox activities, indicating further augmentations both in anaerobic capacity and in the capacity to oxidize fatty acids. The glycolytic pathway represented by PFK, was unaffected by the training regimen m-GPD representing the glycerophosphate shuttle, showed no effects of training.

Pette and coworkers (Bass *et al* 1969 Staudte and Pette 1971) have demonstrated correlations between the activities of enzymes from different metabolic pathways, suggesting a pattern of functional coordination. One of the most prominent correlations is the one between CS and HAD In the present material, this correlation was found before (r 0.7[illegible], $p < 0.01$) and after ($r = 0.81$ $p < 0.001$) the first training period, and at the 60 week biopsy occasion ($r = 0.90$ $p < 0.001$) No significant correlation was, however present before ($r = 0.33$) or after ($r = 0.39$) the second training period, suggesting a loss of coordination.

Studies on mixed human muscles with differing fibre composition, and on individual muscle fibres (Essén *et al* 1975 Sjödin 1976, Thorstensson 1976) have shown that slow twitch and fast twitch fibres differ in their metabolic profiles. The present data failed to reveal any relationships between fibre composition and enzyme activities, with the exception of LDH versus %ST after period I ($r = -0.62$, $p < 0.05$) and m-GPD versus %ST before period II ($r = -0.70$ $p < 0.01$).

The muscle protein content changed in response to training and inactivity and was 11.4 ± 0.5 (16) 13.4 ± 0.9 (16), 11.6 ± 0.6 (16) 14.1 ± 0.7 (16) and 9.3 ± 0.6 (7) (per cent of wet weight means ± S.E. number of subjects within brackets) at the start of the trial, after period I after inactivity after period II and after period III respectively The protein content was significantly higher after period II than before periods I and II The last (60 week) value was significantly lower than all the other values. This was not due to a low protein content in the 7 subjects before period III (mean value 13.7%)

Muscle ultrastructure Data on volume fraction, number per unit volume or sarcomere, and mean volume of mitochondria as well as the volume fraction of lipid droplets before and after each training or inactivity period are given in Fig. 2.

The only observed ultrastructural effect of the first training period was a doubling of the intracellular triglyceride content. This was, however restored to pretraining levels after the inactivity period, and no corresponding increase was observed during the subsequent training periods. During the second training period, the mitochondrial density in the fibrillar space tended to increase ($p < 0.1$) and at 60 weeks it was significantly greater than before training. This increase seemed to be due mainly to a rise in mitochondrial number indicated by a tendency towards an increased mitochondria-to-sarcomeres ratio (N_a, Fig. 2, lower left).

Essentially the data showed no clear-cut effects of the training regimen on subsarcolemmal mitochondria. This may be a true phenomenon, but no definite conclusions can be drawn because of the relatively sparse occurrence of subsarcolemma zones in the micrographs resulting in a small sample.

A correlation between mitochondrial density in the fibrillar space and cytox activity was present after the first ($r = 0.53$ $p < 0.05$) and second ($r = 0.64$ $p < 0.05$) training periods.

Smokers vs. non-smokers. Before training period I smokers ($n = 8$) had significantly lower cytox activities (2.60 ± 0.26 *vs.* 4.0? ± 0.32 μmol O min (g wet wt) means ± S.E.

—dtaneous, parallel variations in intracellular triglyceride content (Fig. 2) might, however a clue to a possible explanation. These effects might be indicative, not of a large training ... increase in the capacity to utilize fatty acids, but of a substrate induction phenome..., due to extensive mobilization of fatty acids from adipose tissue in response to the un-...ar exercise stress. This view is supported by the fact that a high-fat diet causes similar ...es in human skeletal muscle (Essén and Jansson, personal communication). Such a ...omenon may be transient, thus explaining the lack of a similar response during the ...ond training period. The lack of an increased activity of HAD as during period II does ...ever not exclude an augmented fatty acid oxidation capacity (Byhund *et al.* to be pub-...ed), since other factors than the β-oxidation enzymes, such as membrane passages, may ...rate-limiting.

In most studies in the literature, the activity of LDH is not influenced by endurance-type ...ning (for review see Gollnick and Hermansen 1973). In the present study increases were ...erved after every training period, suggesting a elevated anaerobic capacity (Fig. 1). This ...crepancy may be due to at least two factors. Firstly the pre-training LDH activities were ...usually low. The same observation has been made by Bass *et al* (1976), who found signifi-...ntly lower LDH activities in the *vastus lateralis* of sedentary men than in "sporting" or ..."thletic" groups. Secondly the training programme contained exercises that presumably ...ased the anaerobic work capacity namely calisthenics and, in the subjects who were ...examined after period III, strength training. Strength-trained athletes tend to have higher ...DH activities in their muscles than untrained subjects (Karlsson *et al* 1975).

Cytox activity was unaffected by the first seven weeks of training, but rose significantly ...ring the other training periods (Fig. 1). This course of events was paralleled by the mito-...ondrial density in the fibrillar space (Fig. 2), indicating a close mitochondrial structure-...ction relationship. These observations are in accordance with a previous study (Kiessling *et al* 1974), where the *cytochrome oxidase activity increased only 9% during eight weeks* ...f moderate training, whereas seven additional weeks produced a further 65% increase. ...hese results suggest the necessity of a rather long period of moderate training to produce ...hanges in mitochondrial oxygen consumption capacity. During the "lag period" extra-...nzymatic changes in favour of an increased aerobic metabolism may occur. These changes ...ay include improved blood supply by circulatory adjustments and increased capillarisa-...tion (Andersen 1975), and augmented intracellular oxygen supply by a rise in myoglobin ...ontent (Pattengale and Holloszy 1967). Persistence of such changes over the inactivity ...eriod would provide an explanation for the difference between the first and second training ...eriods.

Improved aerobic conditions during prolonged muscular work may be an essential factor ...or the induction of aerobic enzymes like cytochrome oxidase and for the increase in ...mitochondrial density. Aerobic conditions have been shown to favour the production of the ...eart specific LDH subunit (Dawson *et al* 1964) and the synthesis of inner mitochondrial ...membrane components in heart and liver of newborn rats (Hallman 1971). Furthermore, it ...is conceivable that slight hypoxia during muscular work due to chronic carbon monoxide ...poisoning may explain the fact that the smokers in this study had lower cytox activities and ...mitochondrial densities than non-smokers before the training started. The disappearence of

power during training periods I and II (r = 0.23). The reason for this could be the relatively low training intensity and the small amount of training carried out in this study. The increase in maximal aerobic power was only about 6 % in each training period, which is about the same as in the corresponding study of Kilbom (1971) but less than expected from other training studies with corresponding length of training period and age range but higher training intensity (*e.g.* Hartley *et al.* 1969). Since the training intensity rarely taxed the oxygen transport system above 80% of maximum and about one third of the training sessions were aiming to increase muscle power rather than maximal aerobic power, variations in individual training intensity and attendance as well as other factors may play a more important role in this investigation as compared to other studies (*e.g.* Hartley *et al.* 1969).

It is also interesting to note that although it has previously been shown that individuals with the lowest pretraining values attain the greatest increase in V_{O_2} max during a short-term training programme (summarized in Saltin *et al.* 1969), the increase in V_{O_2} max in the present study was not correlated to the pretraining value. This may again be explained by the fact that the amount of physical training carried out was fairly small, not utilizing the whole increase potential in maximal aerobic power present in untrained individuals. In this respect, there were no differences between individuals, who never had been physically active and those, who more than 10 years ago had been engaged in regular physical training.

Seven weeks of low intensity training was evidently not enough to prevent the oxygen transport system from returning to pre-training values during 8 weeks of inactivity. The seven weeks of renewed physical training increased maximal aerobic power to the same extent as did the first training period for the group as a whole, however with a large individual variation (*cf.* above). Thus, the previous training period did not influence the effects of the second training period on maximal aerobic power.

Muscle metabolism and ultrastructure. The low-intensity training regimen employed was clearly sufficient to elicit significant metabolic adaptations in the *m. vastus lateralis*. The pattern of adaptation differed considerably between the three periods of training, a fact that appears to be due to (a) the duration of the training period and (b) effects of the previous training period.

Firstly, the much more pronounced changes over a long period of low intensity training as opposed to a short one are obvious when comparing the different training periods (Fig. 1 and 2). Adaptation to prolonged low intensity training thus seems to be a gradual, long term process as far as skeletal muscle metabolic capacity is concerned. There were, however, some interesting short-term effects that will be discussed later on.

Secondly, some of the differences in training response between the identical first and second training periods may possibly be due to persisting effects over the inactivity period.

Several investigations have demonstrated an increased fatty acid oxidation capacity after training (*e.g.* Baldwin *et al.* 1972, Molé and Holloszy 1970, Molé *et al.* 1971). In the present study increases in the activity of the β-oxidation enzyme HAD were observed twice (Fig. 1). The rise during the last, prolonged training period is in concert with the elevated overall oxidative capacity and probably reflects a significant increase in fatty acid combustion capacity. The significance of the 58 % increase during the first training period is somewhat more obscure, since it did not reoccur when training was resum[illegible] [illegible] f[illegible]er inactivity. The

ous, parallel variations in intracellular triglyceride content (Fig. 2) might, however e to a possible explanation. These effects might be indicative, not of a large training- crease in the capacity to utilize fatty acids, but of a substrate induction phenome- to excessive mobilization of fatty acids from adipose tissue in response to the un- exercise stress. This view is supported by the fact that a high-fat diet causes similar in human skeletal muscle (Essén and Jansson, personal communication). Such a non may be transient, thus explaining the lack of a similar response during the aining period. The lack of an increased activity of HAD as during period II, does not exclude an augmented fatty acid oxidation capacity (Bylund *et al* to be pub- nce other factors than the β-oxidation enzymes, such as membrane passages, may miting.

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these differences during the training also points to improved aerobic conditions as being a result of the training.

An increased muscle protein concentration in response to training was observed after the second training period This has previously been seen in humans (Bylund *et al.*, to be published) but not in rats (Askew *et al* 1973 Winder *et al* 1974). In contrast to this, the protein content was lowered after the prolonged training period, which partly consisted of strength type training. Hypertrophy due to chronical overload has been noted to cause a decrease in protein concentration in rat skeletal muscle (Ianuzzo and Chen 1976)

Slow and fast twitch human skeletal muscle fibres differ in metabolic profile, as revealed by single fibre analyses (Essén *et al* 1975 Sjödin 1976, Thorstensson 1976). These fundamental differences may give rise to significant correlations between the percentage of a fibre type and *e.g* enzyme activities in mixed muscle samples with a range of fibre composition (*e.g* Taylor *et al* 1974 Thorstensson 1976) In the present material two such correlations were found (LDH *vs* %ST after the first and m-GPD *vs* %ST before the second training period) Since most metabolic parameters are susceptible to environmental influences such as training, individual variations in these respects probably obscure the fibre type dependence in most cases.

Systemic vs local adaptation The results of the present investigation apparently suggest a separation of systemic and local training effects The data from training periods I and II show the same improvement in oxygen uptake whether there is an increased skeletal muscle oxidative capacity or not The separation is even more obvious during period III, with a large increase in muscle metabolic capacity but no change in maximal aerobic power It thus appears possible, by low intensity training, to increase the oxygen uptake capacity to some extent without a simultaneous change in the oxidative potential of the working muscles. Conversely it appears possible to increase muscle metabolic capacity without a change in maximal oxygen uptake. In view of these observations, no direct causal relationship seems to exist between maximal oxygen uptake and muscle metabolic capacity in sedentary or moderately trained men

It does not seem undue to speculate that the primary factor limiting the maximal oxygen uptake during running in sedentary individuals might be associated with the supply of oxygen to the working muscle cells during training, improved oxygen delivery together with the increased energy demands then may induce an elevated muscle metabolic capacity which in turn calls for an improved function of the oxygen transport system, and so forth.

Since the increased muscle oxidative potential observed after training periods II and III was not associated with an increase in maximal aerobic power its significance must be sought elsewhere. Most probably this adaptive response is related to an improved endurance enabling the muscles to work longer at a submaximal level by shifting the energy metabolism towards a greater fatty acid utilization, thereby sparing muscle and liver glycogen stores.

As already mentioned, the low intensity training used in this study brought about only a fraction of the possible increase in maximal aerobic power Apart from training intensity attendance and related factors, the increase potential is undoubtedly dependent upon the individual genetical endowment Consequently one might expect those with the highest proportion of oxidative (ST) muscle fibres to attain the greatest increase in oxygen uptake

raining programme. This was actually the case during the first training period, but ods II or III or the various periods together were considered, no such relationship established. Thus, during low intensity training, factors other than the genetical ation are ultimately decisive for the actual change in aerobic power. With an in-raining intensity a more prominent correlation between the muscle fibre compos-the increase in maximal oxygen uptake might be expected.

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Structural "Resetting" of the Coronary Vascular Bed in Spontaneously Hypertensive Rats

By

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nce and functional importance of the "structural autoregulation" which charac
e precapillary resistance vessels in hypertension, has been explored and docu-
oth in man and in rats (*cf* Folkow *et al.* 1973 1974). Basically it constitutes a local
ponse to sustained rises in average tissue load, and in the resistance vessels it takes
of rapidly established media thickening once the average arterial pressure starts to
ally the media hypertrophy is associated with narrowing of the inner radius, so
uctural increase in wall radius ensues. The same principle of tissue adaptation affects
systemic arteries and the left heart. Whereas the rebuild of the resistance vessels
tes to an increased flow resistance, that of the systemic arteries contributes to baro-
resetting (*cf* Jones 1977) and the left ventricular hypertrophy to a functionally
t shift of the Frank Starling relationship (Hallbäck, Isaksson and Noresson 1975).
rning the various systemic circuits, the hemodynamic consequences of this structural
on have so far been analysed mainly in the vascular beds of skeletal muscle, gastro-
l tract and kidneys, using spontaneously hypertensive rats (SHR) as a model of
ential hypertension (*cf* Folkow Hallbäck and Noresson 1977 Folkow *et al* 1977).
iled analysis has, however been performed on the coronary vascular bed where the
n must be especially complex since the myocardium simultaneously undergoes a
ophic adaptation. The present study is an attempt to explore in which way the coro-
uit is altered concerning dimensions and reactivity in the course of hypertension by
ng some hemodynamic characteristics of the coronary vascular beds in isolated
rom SHR and normotensive control rats (NCR).

and 15 age-matched NCR with body weight around 350 g were used. The SHR were in the phase
ished hypertension and mean arterial pressure during awake conditions was 158 ± 6 mmHg in SHR
3 mmHg in NCR.

erfusion of the coronary vascular bed was performed in a system for retrograde perfusion of the
edy *et al* 1967) consisting of an oxygenating chamber and two parallel-coupled perfusion pumps.
hese constant-flow pumps perfused the heart and the other one was used to allow the perfusion
(Krebs bicarbonate buffer 100 ml) to be well oxygenated in the chamber. Under nembutal anesthesia
r heparinization the rat hearts were excised and immediately put into ice chilled saline. Within one
) min a steel cannula was inserted in the aorta towards the heart and the blood was thoroughly
out before the cooled hearts were connected to the recirculating system for coronary perfusion.
was measured at the level of the aortic root by a transducer recording system. Flow could be set at

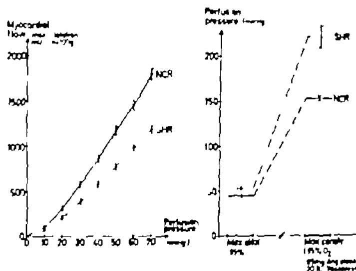


Fig. 1 Compiled results of 15 SHR and 15 NCR. Vertical bars indicate SE. *Left part* illustrates the pressure/flow relationship of the coronary vascular beds of SHR and NCR. At all pressure levels indicated flow is significantly lower ($p < 0.05$) in SHR than in NCR, except at the pressure of 10 mmHg. *Right part* shows the pressure levels at maximal dilatation and maximal constriction at a constant flow of 13.2 ml/g min

any wished level and the resistance of the coronary vascular bed could hence be calculated at different levels of pressure and flow

Initially the perfusion medium was well oxygenated *i.e.* gassed with 5% CO_2 in oxygen and flow was kept at 13.2 ml/g min When a steady pressure level was obtained, supramaximal amounts of vasopressin (20 IU) and of angiotensin II (0 5 mg), were added to the 100 ml perfusate to estimate the maximal pressor response, *i.e.* the maximal contractile strength of the coronary resistance vessels.

After changing the perfusate so that the pressor agents were eliminated, the new perfusate was gassed with 5% CO_2 in nitrogen The coronary vessels were then considered maximally dilated, which was checked by adding adenosine (50 / M) Flow was altered in a random fashion so that the pressure-flow relationship for the completely relaxed coronary resistance vessels could be determined For each heart a curve, relating pressure to flow/g tissue × min, was deduced

During anoxic perfusion and maximal coronary vasodilatation, pressure-flow curves were constructed to characterize the dimensional design of the coronary vascular beds in SHR and NCR and the mean values are presented in Fig. 1 (left panel) As seen from the Figure the SHR curve is shifted to the right, and flow increases relatively less for a given pressure increase than the NCR one. Thus, at any given distending pressure the SHR resistance vessels exhibit a higher resistance per g of myocardium than the NCR ones also at complete relaxation This indicates a structurally determined reduction in internal radius of the SHR coronary vessels Further the SHR coronary resistance vessels are less distensible than the NCR ones which indicates thicker vascular walls in SHR (*cf* Hallbäck, Lundgren and Weiss 1974).

When the hearts were perfused at normal oxygen tension at a constant flow level addition of supramaximal doses of vasopressin and angiotensin caused a significantly greater increase in arterial perfusion pressure in SHR, 169 ± 11 mmHg, than in NCR, 105 ± 13 mmHg ($p < 0.001$) (Fig. 1 right panel) Thus the maximal levels of arterial perfusion pressure reached in SHR was 221 ± 12 mmHg and in NCR 153 ± 3 mmHg, a 45 per cent difference. The ability to produce a greater maximal contractile force suggests the presence of a correspondingly greater bulk of contractile media tissue in the SHR coronary resistance vessels than in the NCR ones, as discussed elsewhere (*cf* Folkow *et al* 1973) Evidently the coronary circuit in SHR exhibits the same type of adaptive structural change as in other systemic circuits.

r the myocardium this has, among other things, the consequence that at the same per a pressure the maximal coronary flow capacity per unit tissue weight is lower in hyper se than in normotensive subjects. This may have serious consequences, particularly if too rapid reduction in mean arterial pressure is induced in hypertensive subjects by macological therapy It also raises the question whether this structural autoregulation e coronary bed can be forced to regress towards normal in the course of a more sustained ure reduction caused by antihypertensive therapy

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Acta physiol scand. 1977 101 366–368

From the Departments of Chemistry and Pharmacology Karolinska Institutet, Sweden

Vasoconstrictor Effect of Thromboxane A_2

By

JAN SVENSSON and BERTIL B FREDHOLM

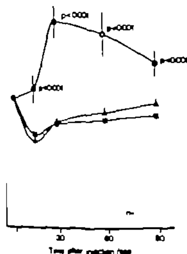
An unstable intermediate in the metabolism of the prostaglandin endoperoxides PGH_2 and PGG_2 was recently discovered (Hamberg *et al* 1975) There is considerable evidence that this compound, termed thromboxane A_2 (TxA_2) plays a role in the platelet release reaction and in platelet aggregation (Hamberg *et al* 1975 Svensson *et al* 1976). It has been shown that this material constitutes the major part of the rabbit aorta contracting substance detected by Piper and Vane (1969) following anaphylaxis in the guinea pig lung (Hamberg *et al* 1975) and is different from the prostaglandin endoperoxides (Svensson *et al.* 1975). When human platelets are caused to aggregate by arachidonic acid or thrombin a substance which causes constriction of coronary artery strips is released. This material appears to be TxA_2 (Svensson and Hamberg 1976, Ellis *et al* 1976). TxA_2 is some 10–100 times more potent than its parent compound PGH_2, in causing constriction of rabbit aorta and swine coronary arteries (Needleman *et al* 1976, Svensson and Hamberg 1976). A role for TxA_2 as a vasoconstrictor following trauma has therefore been proposed (Needleman *et al.* 1976, Ellis *et al* 1976 Svensson and Hamberg 1976) In the present study we have attempted to study the vasoconstrictor effects of TxA_2 under *in vivo* rather than *in vitro* conditions.

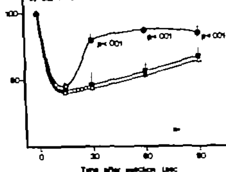
The experiments were performed on 5 cats (2.8–3.7 kg) and 3 dogs (10–13 kg) anesthetized with sodium pentobarbital. Blood pressure and heart rate were recorded by means of a Statham P23AC pressure transducer and a Grass polygraph. Subcutaneous adipose tissue (Rosell 1966) and gracilis muscle blood flows were recorded in dogs by means of arterial drop counters. Mesenteric and femoral blood flows were similarly determined in cats.

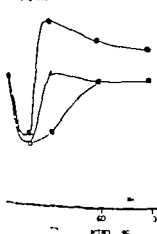
TxA_2 was generated, as described previously (Svensson *et al* 1976), by incubating human platelets (10^9/ml) with arachidonic acid (5 μg/ml) for 30 s at 37 C. The mixture was rapidly filtered through a Millipore filter (0.45 μm). The filtrate was injected close in a volume of 0.3–0.5 ml. For comparison PGD_2, PGE_2, PGH_2, arachidonic acid, ADP and frozen and thawed platelets were similarly injected.

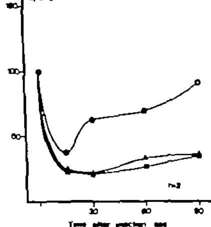
Statistical comparisons were performed using Mann-Whitney U-test.

The main findings of the present study are summarized in Fig. 1 It is evident that platelet filtrates that have been standing for 90 s or more produce profound vasodilatation in all vascular beds studied. This relaxation could be mimicked by frozen and thawed platelets and may therefore be due to some natural constitutent of platelets. One such constituent, ADP, produced a similar vasodilatation in amounts of 2.5–100 nmol. Platelets release about 2









Effect of platelet filtrate on the vascular resistance in) cat mesentery b) cat hindlimb,) dog ..., and d) dog adipose tissue. A washed platelet suspension was incubated with arachidonic acid as ... in methods. After filtration the platelet filtrate was incubated for 0–20 (○—○), 90–150 (△—△) ...–400 (■—■). The vascular resistance 60 after injection of the first (TxA_2-containing) was 96 ... than after the latter (ithout TxA_2) in cat mesentery 60 higher in the cat hindlimb, 97 higher in ... gracilis muscle and 170 higher the dog subcutaneous adipose tissue.

... ADP 10^9 cells during aggregation (S ensson *et al.* 1976) *i.e.* the number of platelets ... in 1 ml incubate. I contrast PGE_1 and PGD had to be given in amounts of 1 μg or ... to produce a similar asodilatation, and arachidonic acid itself produced negligible ...ular effects even in amounts four times as large as those added to the platelet suspension. H (1 μg i.a.) produced a clearcut asodilatation in dog gracilis and adipose tissue and in

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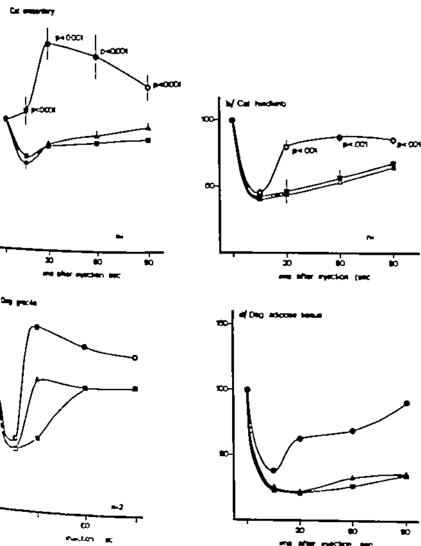
An unstable intermediate in the metabolism of the prostaglandin endoperoxides PGH and PGG was recently discovered (Hamberg *et al* 1975) There is considerable evidence that this compound, termed thromboxane A (TxA_2), plays a role in the platelet release reaction and in platelet aggregation (Hamberg *et al* 1975 Svensson *et al* 1976). It has been shown that this material constitutes the major part of the rabbit aorta contracting substance detected by Piper and Vane (1969) following anaphylaxis in the guinea pig lung (Hamberg *et al* 1975) and is different from the prostaglandin endoperoxides (Svensson *et al* 1975). When human platelets are caused to aggregate by arachidonic acid or thrombin a substance which causes constriction of coronary artery strips is released This material appears to be TxA (Svensson and Hamberg 1976, Ellis *et al* 1976) TxA is some 10–100 times more potent than its parent compound PGH_2, in causing constriction of rabbit aorta and swine coronary arteries (Needleman *et al* 1976 Svensson and Hamberg 1976). A role for TxA as a vasoconstrictor following trauma has therefore been proposed (Needleman *et al* 1976, Ellis *et al* 1976, Svensson and Hamberg 1976) In the present study we have attempted to study the vasoconstrictor effects of TxA under *in vivo* rather than *in vitro* conditions.

The experiments were performed on 5 cats (2.8 3 7 kg) and 3 dogs (10–13 kg) anesthetized with sodium pentobarbital Blood pressure and heart rate were recorded by means of Statham P23AC pressure transducer and Grass polygraph Subcutaneous adipose tissue (Rosell 1966) and gracilis muscle blood flow were recorded in dogs by means of arterial drop counters. Mesenteric and femoral blood flows were similarly determined in cats.

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Effect of platelet filtrate on the vascular resistance in) cat mesentery b) cat hindlimb, c) dog and d) dog adipose tissue A washed platelet suspension as incubated ith arachidonic acid as in methods After filtration the platelet filtrate as incubated for 0–20 (○—○), 90–150 (△—△) 400 (■—■) The vascular resistance 60 after injection of the first (TxA_2-containing) as 96 after the latter (without TxA_2) in cat mesentery 60 higher in the cat hindlimb, 97 higher in gracilis muscle nd 170 higher in the dog subcutaneous adipose tissue.

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In contrast to platelet filtrates that have been left for 90 s or more, filtrates injected immediately caused a significantly smaller degree of vasodilatation and in the case of the cat mesenteric circulation and the dog gracilis muscle actually caused a transient vasoconstriction It is known that the platelet filtrates used contain 266±46 ng TxA_2/ml immediately after filtration (Hamberg and Svensson 1976) TxA disappears with an apparent half time of 30–40 s (Hamberg *et al* 1975) Thus significant amounts of TxA would be present only in the unincubated platelet filtrates while the incubated platelet suspensions would contain 10 per cent of the original amount or less. Thus our findings suggest that filtrates of platelets aggregated with arachidonic acid contain two different types of vaso-activity: a stable vasodilatory material and an unstable vasoconstrictor substance. Since TxA_2 is present in the filtrates, and because it is quite unstable and causes constriction of isolated vascular smooth muscle the possibility is a strong one that the vasoconstrictor material is indeed TxA

Only in two tissues was the net effect of the vasodilatory and the vasoconstrictor material a decrease in flow and even in these it was moderate. This indicates that even though TxA is a potent vasoconstrictor the simultaneous formation of vasodilator metabolites, *i e* ADP and its breakdown products, may antagonize its actions. The net result may be only limited if any vasoconstriction Furthermore, the present data indicate that the effect of materials released from the aggregated platelets may cause different effects on the circulation in different vascular beds

This study was supported part by the Swedish Medical Research Council (04X 2553 03X 17) and Magnus Bergvalls stiftelse.

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Acta physiol. scand. 1977 101 369–381

From the Institute of Medical Physiology A, the Panum Institute,
University of Copenhagen, Denmark

Impulse Dependent Adaptation in *Helix Pomatia* Neurones: Effect of the Impulse on the Firing Pattern

By

MORTEN COLDING-JØRGENSEN

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Abstract

COLDING-JØRGENSEN, M. *Impulse dependent adaptation in* Helix pomatia *neurones: Effect of the impulse on the firing pattern.* Acta physiol. scand. 1977 *101* 369–381

[illegible] of the ventral ganglion of *Helix pomatia* an adaptation type is demonstrated, where the im[illegible] contribute to the adaptation, as if slowly changing outward current were associated to every [illegible]. The current appears to be accumulated from impulse to impulse and the accumulated current to [illegible] towards zero in the interval between the impulses. This type of adaptation is called *impulse dependent*. A method to distinguish between impulse dependent adaptation and impulse independent [illegible] is described. Typical adaptation curves for the impulse dependent adaptation are shown. [illegible] strong adaptation there is linear relation between the steady state frequency and the applied [illegible]. When stimuli of short duration are applied repetitively neurones with impulse dependent [illegible] respond with spikes on an increasing fraction of the stimuli as the stimulus strength is [illegible]. A simplified model of the adaptation is proposed, and the firing pattern of the cells is compared with that of the model. In this way numerical values of the model parameters have been estimated.

[illegible] cells and sensory cells are able to transform an outer action into a collection of [illegible] potentials, where the timing or rhythm of the action potentials in some way is related [illegible] the action. The mechanisms, which are responsible for this transformation—called the [illegible] process, are, however, only sparsely known. A way of elucidating these mechanisms [illegible] to apply different, known stimuli to the nerve cell and to observe the resulting changes in [illegible] activity of the cell. Many nerve cells will thus start firing with a high firing frequency [illegible] a stimulus suddenly is applied to the cell, but the firing frequency decreases with time, [illegible] the stimulus is continued. This phenomenon is called adaptation.

While the mechanisms responsible for the nerve action potential itself have been [illegible] intensely for the past 3 decades (Hodgkin and Huxley 1952, Dodge 1963, Franken[illegible] and Huxley 1964, Connor and Stevens 1971), only a few attempts have been made [illegible] clarify the mechanisms responsible for the firing interval. Furthermore, since Hodgkin's [illegible] description of the different adaptation types in *Carcinus* axons, most work on adapta[illegible] has been a phenomenological description of the firing behaviour (Eyzaguirre and [illegible] 1955, Fuortes and Mantegazzini 1962, Iggo and Muir 1969, Nakajima and Onodera

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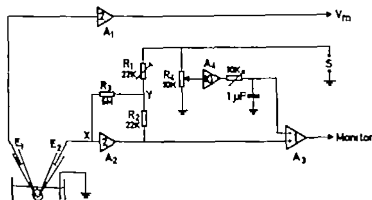
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1 Outline of the experimental setup. A_1 and A_4 are amplifiers with high input resistance, capacity neutralization and gain of 2. A_2 is coupled as current generator where the current, I_m, through the electrode is proportional to the potential S as described in the text. The membrane potential is recorded by the amplifier A_1, but when only one electrode is used the membrane potential is monitored by the amplifier A_3.

... connected directly to ground. With the potential S at zero R can be adjusted so no bias current is passed through the cell.

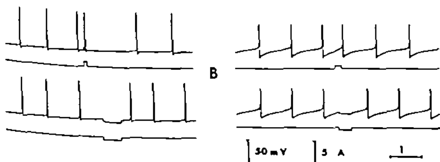
In some of the experiments only electrode E_2 was inserted into the cell. The membrane potential was then monitored by amplifier A_3, where a potential proportional to the current through the cell was subtracted from the output of A_2. By adjusting the resistor R_4 and slowing the output of A_4 a little by the own RC-network the potential drop over the electrode could be cancelled out, at least when the current was of the order of a few nA or less.

The cells used in the experiments were selected at random, mainly on the surface of the posterior part of the ganglion.

Results

Demonstration of impulse dependency

The cells usually fired with a burst of activity when the electrodes penetrated the membrane. Some of the cells became silent, whereas others continued to fire with a constant frequency. A hyperpolarizing or depolarizing current was led through the current electrode



Discrimination between cells with impulse dependent (A) and impulse independent (B) types of ... For each cell is shown two recordings consisting of an upper trace displaying the intracellularly recorded membrane potential (monitor) and a lower trace displaying the current passed through the electrode. Single electrode experiment.

1969 a, b Granit 1972, Connor 1975), distinguishing between fast and slow adaptation and between tonic and phasic behaviour For the cat motoneurone, however it has been shown (Kernell and Sjöholm 1973 Baldissera and Gustafsson 1974 Gustafsson 1974 that the adaptation is accompanied by an increase in the potassium conductance, and this increase can account for the adaptation of the neurone. Similar conductance changes have been demonstrated in several neurones (Jansen and Nicholls 1973 Gola 1974 Barret and Barret 1976) Recently a model for the adaptation of neurones from *Archidoris montereyensis* and *Anisodoris nobilis* has been presented by Partridge and Stevens (1976), where the properties of the conductance were revealed through a voltage clamp technique

In the present paper it is shown that the mechanisms governing the firing interval can be elucidated further by applying transient stimuli to the cell In this way it is possible to distinguish between two apparently fundamentally different mechanisms. In one type of cells the firing interval seems to depend solely on, *when* preceding impulses were elicited and not on *how* they were elicited This type of behaviour is called *impulse dependent*. In another type of neurones the firing appears to be almost completely independent of the presence of preceding impulses. This firing is called *impulse independent* or since it in some way must be related to the stimulus itself *stimulus dependent* The impulse dependent type is most easily understood if it is assumed that every impulse has the same effect on the firing period, that the effect is accumulated from impulse to impulse, but that it is limited in time. The effect may be due to an outward current associated to every impulse, and in the appendix a model is outlined, based on this possibility By comparing the firing of the cells with the model important parameters of the cells are estimated Preliminary reports have previously been given on the impulse dependent and stimulus dependent adaptation type (Colding-Jørgensen 1975 a, b 1976 a)

Methods

The ventral ganglion of *Helix pomatia* was isolated, the covering connective tissue was removed, and the ganglion was placed in a chamber containing a solution of the following composition (mM): NaCl [illegible] KCl 4 $CaCl_2$ 7 $MgCl_2$ 5 Tris-Cl 5 (pH 7.5). Cells were impaled with glass microelectrodes filled with 3M KCl and with resistance of 5-15 MΩ

The experimental setup is shown in Fig 1 Amplifier A and A_2 are preamplifiers with high input resistance, capacity neutralization, and with a gain of 2. The microelectrode E_1 was used to record the membrane potential of the cell, while the electrode E_2 was used to inject current through the cell membrane. The amplifier A_2 was used to control the current through the cell so it became independent of the resistance of the electrode E_2 and of the cell membrane potential. This can be illustrated in the following way With the potential X at the input the output of A_2 is 2X. If the resistances of the resistors [illegible] and R_2 are equal, and if the resistance of R_2 is much larger than R_1 and R_p, then the potential, Y on the right hand side of R will be

$$Y = \frac{1}{2}(S+2X) = \frac{1}{2}S + X,$$

where S is the potential applied at the terminal S The current, I_m, through R is therefore

$$I_m = (Y-X)/R = S/2R_p$$

which is independent of X. Since the input resistance of A and A is high, the current I_m must flow through the cell membrane to ground. With R_p equal to 5 MΩ the current [illegible] 100 nA per volt applied at [illegible] Further measurement of the current is not necessary and consequently the fluid of the chamber [illegible]

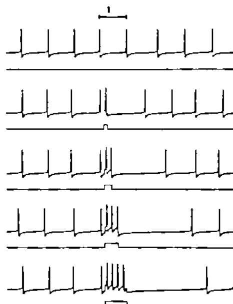


Fig. 4 Reaction of cell with impulse dependent adaptation on an increasing number of impulses. The upper trace of each recording is the membrane potential (monitor) and the lower trace the current through the cell. Single electrode experiment

...ed through the cell, thereby eliciting 1 2, 3, or 4 extra spikes. As the number of spikes was increased, the firing period just after the stimulation was prolonged more and more, whereas the period following the prolonged period was unaffected. The prolongation was almost proportional to the number of spikes each spike increasing the firing period by 0.57 s. Impulse dependent adaptation is thus additive.

Response to step of constant current

In order to demonstrate the adaptation of the cells depolarizing current steps were passed through the membrane of the cell, and Fig. 5 shows the corresponding recordings of the membrane potential for steps of 0.2, 0.4 and 0 75 nA. The onset of the current can be seen as a small deflection from the resting potential in the beginning of each recording. The figure shows. (1) The adaptation occurs for small currents after the first few spikes thus the effect of a single impulse is strong. To a current of 0.2 nA the cell adapts completely after one spike. (2) The threshold potential does not change during the firing. The cell

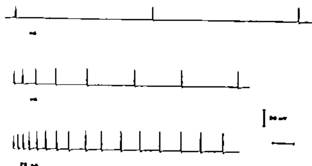


Fig. 5. Firing pattern of the impulse dependent type in response to current steps of different magnitude (experiment with two electrodes).

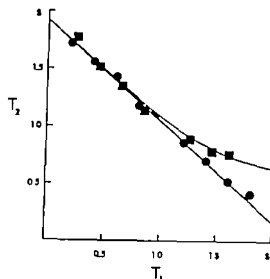


Fig. 3 Relation between the induced firing peri[od] T and the subsequent firing period, T as describ[ed] in the text. ●—cell 1 ■—cell 2. The curve [for] is draw according to eq. (6) in the appendix w[ith] the same parameters as for the curves in Fig. Through the points for cell 1 is drawn a straight lin[e]

to make the cell fire at a constant frequency usually 0.3–1 Hz. Some of the cells produc[ed] only phasic activity these cells were not used. The cells were tested for impulse dependenc[e] as shown in Fig. 2. The two upper sets of recordings, A, are from one cell the lower, I from another cell The current was adjusted to make the cells fire with a period between 1 0 and 1 1 s, and an additional rectangular current pulse was applied as show[n] on the figure. The two cells behaved differently In A the introduction of an extra spi[ke] prolonged the subsequent period the delay of the spike shortened it. In B only the period wi[th] the stimulus was affected. Thus the behaviour of the cell in A was modified by the presen[ce] of an impulse, the behaviour of the cell in B was not. The procedure distinguished reliab[ly] between impulse dependent and impulse independent behaviour and was therefore we[ll] to identify cells with impulse dependent adaptation

Of the 147 cells investigated 81 or 55%, displayed impulse dependent firing, and 50-60% of these fired spontaneously Stimulus dependent behaviour was found in 52 cells (35 of which 21 (14% of the total) displayed phasic activity The remaining 14 cells (10% showed only weak impulse dependency or no definite adaptation.

The impulse dependent behaviour can be elucidated further With the stimulus applied a[t] different times after a spike, thereby varying the duration, T from the normal spike to th[e] induced, the subsequent firing period, T_2, depends on T This is demonstrated in Fig 3 The figure shows the relation between T and T for two cells with impulse depende[nt] adaptation. Each symbol represents an average of 5–15 samples with the same T and wi[th] a S.E. less than the size of the symbols. In all cases the mean value of the steady firin[g] period, T was 1.05 s (limits 1 0 and 1 1 s). For values of T less than T both cells gave fairly linear relation between T and T_2, but for T larger than T the two cells behaved in slightly different way Cell 1 (circles) continued to give a linear relation, whereas th[e] relation for cell 2 (squares) seemed to approach a constant value for T_2, which was rathe[r] large. It was not possible, even with very large values of T to obtain a T below 0 7 s.

Another way of demonstrating impulse dependency is illustrated in Fig. 4 The cell wa[s] firing with a frequency close to 1 0 Hz, when an additional rectangular current pulse wa[s]

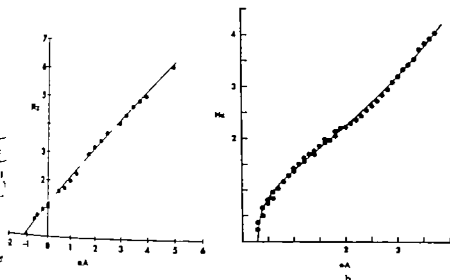


Fig 7 Relation between the constant current stimulus and the resulting steady state firing frequency a Linear relation for cell with strong adaptation b S-shaped relation for cell with weak adaptation. The curved line drawn according to eq (4) of the appendix Abscissa, current stimulus in nA, ordinate firing frequency in Hz.

spontaneously occurring EPSP's the cells were stimulated by current pulses of 10–300 ms duration at a constant frequency of between 0.5 and 2 Hz. Such patterns of EPSP's was predominant in the ganglion. Driven in this manner the cell frequently failed to respond even to large stimuli, and the failures became more frequent the faster the cell was driven, the smaller the stimulus strength, and the shorter the pulse duration. The behaviour is shown in Fig 8 a. The cell was driven at a frequency of 1.75 Hz with current pulses of 10 ms duration and increasing stimulus strength. The cell responded with an increasing number of spikes as the current strength was increased, until it finally responded to every stimulus at a stimulus strength of 7 nA. The cell acted in this way as a frequency converter where the conversion ratio depended on the stimulus strength. In addition Fig. 8 a shows that even though the cell did not respond to every stimulus, its firing was very regular The cell did not only respond to 33 %, 50 % or 67 % of the stimuli it responded on every third, every second or failed on every third respectively

The behaviour may be understood by inspection of Fig. 8 b. The recording is from the same cell, but on a different time scale with a stimulus duration of 100 ms and an amplitude of 0.55 A to enhance the effect. After a dropout the spike is delayed more and more with respect to the stimulus, as if the threshold were increasing during the firing. When the threshold becomes too large, the stimulus fails to excite the membrane, and a new dropout occurs. During this dropout (dropouts) the threshold decreases, until the stimulus again is sufficient to excite the membrane. The relation between the conversion ratio and the stimulus strength for the recordings in Fig. 8 is shown in Fig. 9 The number of spikes elicited per stimulus is plotted as a function of the stimulus strength. A straight line is drawn through the points indicating that the relation is fairly linear

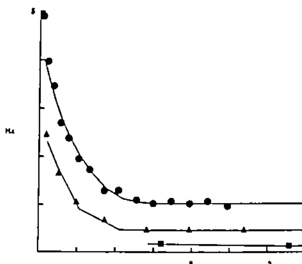


Fig. 6. Adaptation curves for the firing shown in Fig. 5. Abscissa: Time since the first spike in seconds, ordinate: instantaneous firing frequency in Hz. ■ – ■ 0.2 nA, ▲ – ▲ 0.4 nA and ● – ● 0.75 nA. The curves are drawn according to the model as given in equation (3) of the appendix.

fires, whenever the threshold is reached, even if the potential changes slowly. (3) The firing is fairly regular even for long firing periods. The cells were able to fire steadily with firing periods as large as 5–15 s. (4) The amplitude of the action potentials increases in the beginning of each recording. This was frequently although not always, seen especially in cells, which had been hyperpolarized prior to the current step to prevent firing.

The instantaneous firing frequencies, the reciprocical of the period between a spike and its precedent, for the three recordings of Fig. 5 are plotted in Fig. 6 as functions of the time from the appearance of the first spike. The symbols represent the frequency measured from the recordings, and the curves are drawn according to the model (*cf.* the appendix). In the beginning of the current step the frequency is falling rather fast from a high value, whereafter it suddenly becomes constant. This "break adaptation" is typical for the impulse dependent firing, especially for small stimuli. In cells, where the adaptation was strong, the break could be very pronounced: the cell fired a few spikes close to each other at the onset of the stimulus, and subsequently the frequency fell to a steady value.

Thus, when the stimulus current is maintained, the frequency becomes constant, and a relation between this steady state frequency and the current stimulus can be obtained. Such relations are shown in Fig. 7 a and 7 b. For a cell with a strong adaptation the relation is almost linear over a considerably large stimulus interval (Fig. 7 a), whereas the relation for a cell with a weak adaptation is s-shaped (Fig. 7 b). Notice that the cell in Fig. 7 a was firing even when no current was passed through the cell and that it had to be hyperpolarized to prevent firing. Such spontaneous activity was frequently seen, but there seemed not to be any detectable difference in the behaviour of the active and silent cells apart from the spontaneous activity. Through the points of Fig. 7 a is drawn a straight line with a slope of 1.0 Hz/nA, while the curve in Fig. 7 b is drawn according to the model (*cf.* the appendix).

C. *Response to repetitive stimulation*

Neurones are normally excited by EPSP s (Excitatory postsynaptic potentials) acting as short current pulses either occurring repetitively or as single pulses. In order to imitate the

pulses through the current electrode. If the stimulus was too small or the delay too short a stimulus failed to excite the cell, and if the stimulus was too large or the delay too long, an impulse would be triggered by every stimulus thereby giving rise to perpetual activity of the cell. However in some interval only a finite train of spikes would appear and with a constant value of the delay and the stimulus strength, the number of spikes elicited would be a constant too. In this way they could reliably multiply their firing frequency by a given number, thus giving rise to conversion ratios larger than 1

Discussion

The results seem to demonstrate that the presence or absence of an impulse can modify the firing pattern This is most clearly demonstrated in Fig. 2 A. It is not likely that the current stimulus itself should cause the behaviour because the pattern was independent of the strength and duration of the stimulus as long as only one impulse was elicited. Moreover, the same behaviour was observed in spontaneously firing cells, when small irregularities occurred in the firing rhythm, and also in these cases a linear relation between the firing period before (T_1) and after (T_2) the disturbance could be found. It is also unlikely that the cell was driven by a regular synaptic input Such activity could drive the cell at a constant frequency but the frequency would be fixed. The firing frequency of the cells could, however easily be changed by passing constant current through the cell, and when the cell was hyperpolarized to prevent firing, no regular synaptic activity could be detected For the driven activity the relation between T and T would be linear but the slope should have been exactly −1 because the sum T + T should be equal to the steady firing period, T The numerical value of the slope was, however always less than unity and it decreased, when T was increased.

Both cells (A and B of Fig. 2) displayed definite adaptation, so the mechanisms, which governed their repetitive behaviour must have been different. The experiments show that the cell with impulse dependent adaptation could act as a stable oscillator which could preserve an almost constant mean frequency in spite of disturbances. It was also able to display dynamic activity in response to a change in the stimulus. A rapid increase in the stimulus strength produced a large transient increase in the firing frequency but only a small increase in the steady frequency In this way the cell was able to transmit information about transient phenomena and superimpose it on a stable firing. In contrast the cell in Fig. 2 B acted as an excellent follower which easily shifted its firing back and forth in response to a disturbance, but the firing period after the disturbance was unchanged.

The effect of an impulse on the firing seems to be additive, which means that the effect of a new impulse is added to the effect of previous impulses (Fig. 4). The effect is, however limited in time (Fig. 2 and 4). After a disturbance the cell resumes its steady firing with the same firing period as before the disturbance.

The repetitive stimulation

In the experiment with repetitive stimulation the stimulus strength was changed, while the steady level of the current was unchanged. A similar firing pattern could have been obtained by using current pulses of the same size and then change the steady level of the

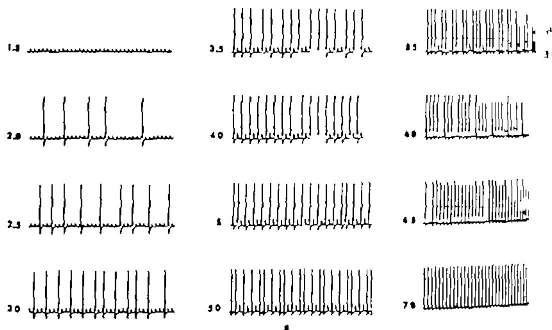
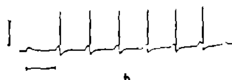
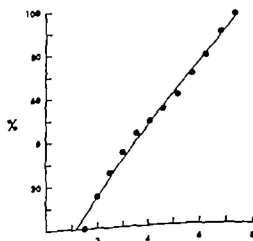


Fig. 8 Response to repetitive stimulation. a. Intracellular recordings from a cell ith impulse depend daptation stimulated with current pulses of 10 ms duration at a frequency of 1 75 Hz. At each record is shown the current strength in nA. (Two electrode experiment).



b. Same cell as in Fig. 8 a. The cell is stimulated with current pulses of 0.55 nA and with a duration of 100 ms. Potential scale 50 mV time scale 1 s.

By the presented procedure conversion ratios below 1 were found. The cell was al able to fire with a frequency higher than the input frequency under some circumstanc This possibility was demonstrated in the following way Every time a spike was elicit it triggered an electronic equipment which after a delay applied a rectangular curre



F g. 9 Relation between the stimulus strength an the percentage of responses for the recordings show in F g. 8 a Abscissa Stimulus strength in nA ord nate Number of spikes elicited as percentage of the total number of stim h applied. The straight li indicates that the relation i almost linear

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current The relation between the conversion ratio and the steady stimulus strength would then be the same as in Fig 9 with the sum of the steady current and the current pulses as the abscissa. Within the ganglion the EPSP's do not change as the stimuli of Fig. 8 a, but slowly changing synaptic inputs or changes in the composition of the extracellular fluid may act in the same way as changes in the steady current strength Thus the cell with impulse dependent adaptation is not only able to display the spatial and temporal summation of subthreshold stimuli but also to suppress the effect of suprathreshold stimuli.

The conversion ratio depends on the frequency of the current pulses. For small frequencies the ratio is 1 but for larger frequencies, where dropouts occur the firing frequency becomes constant, as if the cell was stimulated by a constant current of the same strength as the pulses. If the size of the EPSP's is a constant, the cell should then fire at a rate, which is independent of the frequency of the EPSP's. The conversion ratio may exceed 1, for example if an excitatory collateral from the axon is returning to the cell body either directly or through interposed neurones, and although the nature of such a collateral is completely speculative, its possible effect is interesting. Due to a constant propagation velocity and synaptic delay (possibly in interposed neurones) the total delay as well as the stimulating synaptic input would be constant The conversion ratio could, however again be varied by varying the basic, synaptic input to the cell

The observed fraction of the impulse dependent cells (55) in the ganglion was remarkably high and may not be representative for the whole ganglion There might have been a tendency to search for impulse dependent cells at places, where they had been found previously and there may be differences between the superficial cells and the cells in the rest of the ganglion.

A model of adaptation

A possible explanation of the impulse dependent behaviour may be that the impulse is associated with an outward current, called the adaptation current, which tends to hyperpolarize the membrane and thus prolong the firing period. The current may be due to an increase in the potassium conductance or to an electrogenic pump as the most obvious possibilities, but in the simplified model of adaptation presented below nothing is assumed about the nature of the current

The model has the following mean features 1 Whenever the membrane potential passes beyond the threshold, a spike is elicited. 2. The spike generating mechanism and the adaptation mechanism are mutually independent 3 The adaptation is governed by an outward current, where each spike increases the current by the same amount, and the current decays exponentially towards zero with a large time constant Finally the relation between the stimulating current, I_m and the firing period T is assumed to be described by the following expression (Colding-Jorgensen 1976 b)

$$I_m - I \quad I \exp(-T/\tau_K) + I_A$$

where I is the rheobasic current I is a constant and τ_K is the time constant for the potassium conductance participating in the spike formation.

It was found that a spike was triggered whenever a threshold was passed even when the potential changed slowly (Fig 2, 4 and especially 5) This confirms the first assumption. The

average adaptation time constant of 100–2 000 times the duration of the spike makes a distinction between a spike generating mechanism and an adaptation mechanism acceptable. The existence of an adaptation current with an exponential decay is not demonstrated in the present paper. It is only shown that the effect of an impulse is decaying towards zero with time, so the exponential decay can be taken as a simple, mathematical expression, which is able to fit the behaviour of the cells. However the assumption that each spike increases the adaptation current by the same amount seems to be justified by the demonstrated in Fig. 4 and to some extent Fig. 7 a.

It is interesting that the simple model is able to mimic the complex behaviour demonstrated in the previous sections. Furthermore, when the adaptation is strong and its time constant large, the equation above can be simplified to

$$I_m - I_r \quad I_A,$$

in order. Whenever the adaptation current, I becomes smaller than the difference between the stimulating current, I_m, and the rheobasic current, I a new spike is elicited. With this simple expression and using eq. (2) of the appendix to find I most of the impulse dependent behaviour can be understood. Only when the firing period according to this simplification approaches the refractory period of the cell (symbolized by the time constant in the appendix) a more detailed model has to be used. It can be seen from the expression, why the impulse dependent cell type is a stable oscillator even at low frequencies. The adaptation current offers kind of feed-back control of the firing, which ensures that a new spike first is elicited, when I_A is decreased sufficiently no matter how small the difference I_m I_r is. The effect of a disturbance, which for example shortens the firing period, is then that the next firing period is prolonged in such a manner that the mean firing period almost is conserved.

The model shows that the current, I released by a single spike is small. In Fig. 6 a value I° 0.104 nA was used, and in Fig. 7a a value of 0.13 nA was found. If it is assumed that the current is due to an increase in the potassium conductance, the corresponding conductance can be found. The equilibrium potential for the potassium ion is in the order of 20 mV below the resting potential (*cf* Colding-Jørgensen 1977) for the cell, so the 0.13 nA corresponds to an increase in the potassium conductance of some 0.65 10^{-8} mho per spike, or to 10–20% of the resting value of the membrane conductance (resistance of the cell 10-30 MΩ). Thus, although the current of a single spike is small, few spikes fired close to each other can change the membrane conductance considerably.

The model is not able to tell anything about, how the adaptation current is created. An electrogenic pump has been reported to participate in the adaptation of the tonic crayfish stretch receptor (Sokolove and Cooke 1971), but the impulse dependent adaptation in these neurones seems to be caused by an increased potassium conductance as demonstrated in a subsequent paper (Colding-Jørgensen 1977). However any membrane, which gives rise to a constant amount of adaptation current per spike, will display a behaviour which can be mimicked by the model. The impulse dependency is thus a property which can be shared by many membranes with different bases for the adaptation current, but with the same firing behaviour.

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In words: Whenever the adaptation current, I becomes smaller than the difference between the stimulating current, I_m, and the rheobasic current, I_r, a new spike is elicited. With this simple expression and using eq (2) of the appendix to find I_A most of the impulse dependent behaviour can be understood. Only when the firing period according to this simplification approaches the refractory period of the cell (symbolized by the time constant in the appendix) a more detailed model has to be used. It can be seen from the expression, hy the impulse dependent cell type is a stable oscillator even at low frequencies. The adaptation current offers a kind of feed-back control of the firing, which ensures that new spike first is elicited, when I_A is decreased sufficiently no matter how small the difference I_m I_r is. The effect of a disturbance, which for example shortens the firing period, is then that the next firing period is prolonged in such a manner that the mean firing period almost is conserved.

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Appendix

The model

A thoroughly description of the model is given elsewhere (Colding-Jorgensen 1976 b). It has the follow three main features. 1 Whenever the membrane potential passes beyond the threshold, an action potential or spike, is elicited. 2. It is possible to distinguish between a spike generating mechanism and adaptation mechanism, which are mutually independent. 3. The adaptation is governed by an outward current, the adaptation current. Each spike increases this current by the same amount, i_A and the current decays exponentially towards zero with a large time constant τ_A

According to the model the i terval, T between two successive spikes is given by

$$I_m - I_0 = I \;\exp(-T/\tau_K) + I_A \quad (1)$$

where I_m is the transmembraneal current, I_0 the rheobasic current, I is a constant, τ_K is the time constant for the normal potassium conductance participating in the spike formation, and I_A is the total adaptive current just before the next spike is elicited. If T_j is the duration of the jth firing period, the corresponding value of the adaptation current, I^j becomes

$$I^j = (i_A + I^j \;)\exp(-T_j/\tau_A) \quad (2)$$

according to the third assumption of the model.

When the membrane starts i a resting state and a current step, I_m, suddenly is passed through the membrane, the successive values of the firing period can be determined from eqns. (1) and (2) as

$$I_m - I \;= I \;\exp(-T_j/\tau_K) + (i_A + I_m - I_0 - I \;\exp(-T_{j-1}/\tau_K))\exp(-T_j/\tau_A). \quad (3)$$

The value T for j=1 must then be set to infinity This relation is used to draw the curves in Fig 6 The parameters used are $I_0 = 0.16$ nA, I $= 2.6$ nA, $\tau_A = 6$ s, $\tau_K = 0.15$ s, and $\gamma = i_A/I = 0.04$.

When the cell is firing with a steady firing period, T eq. (3) reduces to

$$I_m - I_0 = I \;\exp(-T/\tau_K) + \;_A\exp(-T/\tau_A)/(1-\exp(-T/\tau_A)). \quad (4)$$

For $\tau_K < T < \tau_A$ and with a not too small value of $\gamma = i_A/I$ eq (4) can be approximated by

$$I_m - I_0 = i_A(\tau_A \nu - \tfrac{1}{2}) \quad (5)$$

where ν is the steady f ring frequency Such a linear relation between firing frequency and current is seen in Fig. 7 a, where the slope of the line is 1 0 Hz/nA. W th a τ_A estimated for this cell of 7–8 s, eq (5) gives i_A of 0.13 nA. I_0 ca be found from the same equation to -1 08 nA. The negativity of I_0 corresponds to a spontaneously active cell. With respect t the cell of Fig. 7 b the relation between frequency and current is not linear It can, however be reproduced by the relation in eq (4) (curved line of the figure) when the parameters are $I_0 = 0.3$ nA, I $= 6.9$ nA $\tau_A = 10$ s, $\tau_K = 0.28$ s, and $\gamma = 0.002$. The constant is here very low It corresponds to a $\;_A$ of 0.014 nA or ten times less than the estimate of i_A for the cell in Fig. 7 a

In the case, where an extra spike is induced at time, T after spike I_A is, when the next spontaneous spike is elicited after a further time, T_2, found from eq. (2) to

$$I_A = (\;_A + (\;_A + I_A)\exp(-T_1/\tau_A))\exp(-T_2/\tau_A),$$

where I_A is the value of I_A at the steady f ring. Combining the equations (1), (7) and (4), the relation between T and T becomes

$$\exp(-T/\tau_K) + \gamma\exp(-T/\tau_A)/(1-\exp(-T/\tau_A))$$
$$= \exp(-T_2/\tau_K) + \gamma\exp(-(T \;+ T_2)/\tau_A)/(1-\exp(-T/\tau_A)) + \gamma\exp(-T_2/\tau_A).$$

With the same approximations as in eq. (5), and for $T \leq 1.8T$ and $T < 0.25\tau_A$ the relation between T and T in eq (6) is approximately linear with a slope of $-1/(1+T/\tau_A)$. This is seen for cell 1 in Fig. The slope of the line drawn through the points is 0 86, so according to the model the time constant τ_A should be 6.5 s. The relation for cell 2 is not linear but can be approximated, as shown on the figure, with the relation i eq (6) with the same parameters as used in Fig. 6.

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Appendix

The model

A thoroughly description of the model is given elsewhere (Colding-Jørgensen 1976 b). It has the follow three main features 1 Whenever the membrane potential passes beyond the threshold, an action potential or spike, is elicited 2. It is possible to distinguish between a spike generating mechanism and an adaptation mechanism, which are mutually independent. 3 The adaptation is governed by an outward current, the adaptation current. Each spike increases this current by the same amount, i_A and the current decays exponentially towards zero with a large time constant τ_A

According to the model the interval, T between two successive spikes is given by

$$I_m - I_e = I \quad \exp(-T/\tau_K) + I_A$$

where I_m is the transmembraneal current, I_e the rheobasic current, I is a constant, τ_K is the time constant for the normal potassi m conductance participating in the spike formation, and I_A is the total adaptation current just bef re the next spike is elicited. If T_j is the duration of the jth firing period, the corresponding value of the adaptation current, I^j becomes

$$I^j = (i_A + I^{j \quad 1}) \exp(-T_j/\tau_A)$$

according to the third assumption of the model.

When the membrane starts in resting state and a current step I_m, suddenly is passed through the membrane, the successive values of the firing period can be determined from eqns. (1) and (2) as

$$I_m - I_e = I \quad \exp(-T_j/\tau_K) + (i_A + I_m - I_e - I \quad \exp(-T_{j-1}/\tau_K)) \exp(-T_j/\tau_A).$$

The value T for $j = 1$ must then be set to infinity This relation is used to draw the curves in Fig 4 The parameters used are $I = 0.16$ nA, $I = 2.6$ nA, $\tau_A = 6$ s, $\tau_K = 0.15$ s, and $\gamma = i_A/I = 0.04$.

When the cell is firing with a steady firing period, T eq (3) reduces to

$$I_m - I_e = I \quad \exp(-T/\tau_K) + i_A \exp(-T/\tau_A)/(1 - \exp(-T/\tau_A)).$$

For $\tau_K < T < \tau_A$ and with a not too small value of $\gamma = i_A/I$ eq (4) can be approximated by

$$I_m - I_e = i_A(\tau_A \nu - \tfrac{1}{2}),$$

where ν is the steady firing frequency Such a linear relation between firing frequency and current is seen in Fig. 7 a, where the slope of the line is 1 0 Hz/nA With a τ_A estimated for this cell of 7-8 s, eq. (5) gives an i_A of 0.13 nA. I_e ca be found from the same equatio to -1 08 nA The negativity of I_e corresponds to a spontaneously active cell With respect t the cell of Fig. 7 b the relation between frequency and current is not linear It can, however be reproduced by the relation I eq. (4) (curved line of the figure) when the parameters are $I = 0.3$ nA, $I = 6.9$ nA, $\tau_A = 10$ s, $\tau_K = 0.28$ s, and $\gamma = 0.002$. The constant is here very low It corresponds t an i_A of 0 014 nA or ten times less than the estimate of i_A for the cell in Fig. 7 a

In the case, where an extra spik is induced at a time, T after a spike, I_A is, when the next spontaneous spike is elicited after f rther time, T_2, found from eq (2) to

$$I_A = (i_A + (i_A + I_A) \exp(-T_1/\tau_A)) \exp(-T_2/\tau_A),$$

where I_A is the value of I_A t the steady f ring. Combining the equations (1), (2) and (4), the relation between T and T becomes

$$\exp(-T/\tau_K) + \gamma \exp(-T/\tau_A)/(1 - \exp(-T/\tau_A))$$
$$= \exp(-T_2/\tau_K) + \gamma \exp(-(T \quad + T_2)/\tau_A)/(1 - \exp(-T/\tau_A)) + \gamma \exp(-T_2/\tau_A).$$

With the same approximations as I eq (5), and for $T < 1.8T$ and $T < 0.25\tau_A$ the relation between T and T i eq (6) is approximately linear with slope of $-1/(1 + T/\tau_A)$. This is seen for cell 1 in Fig 1. The slope of the line drawn through the points is 0 86, so according to the model the time constant τ_A should be 6.5 s. The relation for cell 2 is not linea but can be approximated, as shown on the f gure, with the relation I eq. (6) with the same parameters as used in Fig 6.

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Impulse Dependent Adaptation in *Helix Pomatia* Neurones: Demonstration of the Adaptation Conductance

By

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Abstract

COLDING-JORGENSEN M *Impulse dependent adaptation in* Helix pomatia *neurones: Demonstration of the adaptation conductance* Acta physiol scand. 1977 *101* 382 393

By conventional voltage clamp methods an increase in membrane conductance after a depolarizing pulse is demonstrated in neurones of *Helix pomatia*. This increase decays exponentially with a time constant in the range of 5–20 s. The rise of the conductance during the depolarization can be represented by 3 exponentials with time constants from 12 ms to 1 s. The steady state value of the conductance depends on the membrane potential in a sigmoid manner. The conductance gives rise to an outward current, and appears to be carried by potassium ions. The firing pattern of the cell is governed by the conductance. A short, rectangular potential change, a pulse clamp, is used to disturb the firing pattern of the freely firing cell. The effect on the firing pattern depends on the height and duration of the clamp pulse in the same manner as the conductance increase. The normal firing produces similar changes in the membrane conductance.

An increase in membrane conductance following spike trains has been demonstrated in *Aplysia* (Brodwick and Junge 1972) in *Helix* (Gola 1974) in leech (Jansen and Nicholls 1973), and in frog motoneurones (Barrett and Barrett 1976) while a slowly changing conductance has been shown to be responsible for the firing period in cat motoneurones (Baldissera and Gustafsson 1971 1974 Kernell and Sjöholm 1973 Schwindt and Calvin 1973 Gustafsson 1974 Mauritz, Schlue, Richter and Nacimiento 1974) The repetitive behaviour of molluscan neurones has been modelled (Connor and Stevens 1971 a, b, c), but these cells displayed no or almost no adaptation. In a recent work however a slowly changing conductance has been demonstrated in the marine molluscs *Archidoris montereyensis* and *Anisodoris nobilis* by Partridge and Stevens (1976) With this conductance included the model of the snail neurone was able to display adaptation resembling the adaptative behaviour of the neurones. It was thus of interest to determine, whether the previously described impulse dependent firing in *Helix pomatia* neurones (Colding Jorgensen 1975 a, b 1976 b, 1977) was associated with a slowly decaying, outward current, and whether this current controlled the firing period as previously assumed (Colding-Jorgensen 1976 c, 1977

methods were used in this investigation. The changes of the membrane conductance studied with a conventional voltage clamp technique. But to examine whether the in membrane conductance was responsible for the firing pattern of the cell another called the pulse clamp method, was used. A rectangular potential shift was imposed cell membrane, while the cell was firing freely. The effect on the firing period of durations and amplitudes of the potential shift was then recorded.

Methods

al ganglion of *Helix pomatia* was isolated and the connective tissue removed. In some cases the tissue was softened with pronase-E applied as concentrated solution for 20 and then away. No difference in the behaviour was seen between treated and untreated ganglia. The was placed in chamber containing solution of the following composition (mM): NaCl 80 $CaCl_2$ 7 $MgCl_2$ 5 Tris-Cl 5 (pH 7.5). The cells were impaled with two glass microelectrodes filled KCl and with resistances of 5–15 MΩ.

up. The experimental setup shown in Fig. 1 was described previously (Colding-Jørgensen 1977), the amplifier A_4 and the analogue switch AS. Amplifier A and A_4 were preamplifiers with resistance, capacity neutralization, and gain of 2. Amplifier A_1 was used to record the potential, V_m, while A_2 was used to inject current into the cell. The current was either con- from the input S or from the output of the high gain amplifier A_4 depending on the position of the switch AS and with current of 100 nA per volt applied. The switch was guided by transistor logical (TTL) inputs with logical 1 close to 5 V and logical 0 close to 0 V and with the switch in position, when C was low (close to 0 V). The setup then worked in current clamp mode with controlled by S and with the membrane potential recorded by A_1. When the input C was high 5 V), the position of the switch was reversed, and the setup worked in voltage clamp mode. membrane potential was recorded by A_1 and fed into the amplifier A_4 together with the command V_c. The current through the cell was then controlled by the output of A_4 and by A_2. The was not measured directly but monitored at the output terminal I_m after passing through suitable filter. The current used to neutralize the electrode capacity was therefore not included the recordings, so the difference between the actual current through the cell and the current at I_m was small and depended mainly on the accuracy of the capacity neutralization. For slow in the current the difference was insignificant.

ctance measurements. The cells were impaled by the current injecting electrode and classified as by Colding-Jørgensen (1977). Only cells with definite impulse dependency were used. The recording electrode was then inserted and the analogue switch shifted, so the membrane was at the resting potential (45 to 60 mV) as preset on the input V

ductance measurements were performed at the resting potential. Before and after conditioning pulses of 10 mV hyperpolarization and 100–200 ms duration were applied, and the current owing to the 10 mV potential change was measured as the difference between the current just start of the pulse and the current just before the end of the pulse (Fig. 3). The conductance calculated as the ratio between the measured current difference and the potential change of the his method should minimize the effect of fast changes in the current trace related both to the of the membrane capacity and to fast, voltage dependent conductance changes. Small depolarizing polarizing potential changes of 1 2 mV produced the same results as the 10 mV hyperpolarization. ulse clamp. With the analogue switch in the current clamp position DC-current was passed the cell and adjusted, so the cell fired with constant frequency between 0.3 and 1 Hz. A TTL-pulse lied to the terminal C at fixed time, T after spike switching the setup to the voltage clamp mode. ay voltage clamp of short duration, called pulse clamp, was applied, where the clamp height ermined by the setting of V and the clamp duration by the duration of the TTL-pulse T avoid ents in the voltage clamp, V was preset so the output of A_4 equalled S. When the clamp was on, V rose towards the desired value with time constant of 0.2 to 1 ms. To accelerate the return the resting potential, when the clamp was turned off, current pulse of 500 nA was added just clamp ended. By adjusting the duration of the pulse the membrane potential was brought back 1 2 ms (Fig. 2).

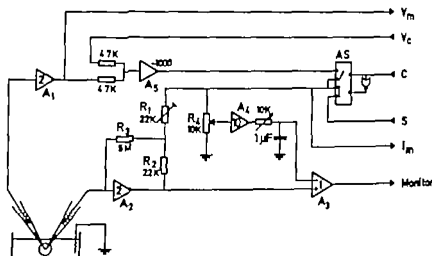


Fig. 1 Outline of the experimental setup.

The time T_p, from the end of the pulse to the beginning of the next spontaneous spike as measur During this period the membrane potential remained close to the threshold for several seconds (cf Fig. A small depolarizing disturbance could thus elicit a spike and shorten T considerably The sum of t disturbed period, T_p, and the following period, T'_p, should, however be almost constant for a cell w strong impulse dependent adaptation (Colding-Jørgensen 1977), so T was found as

$$T = T' + T' - T$$

because T should have been equal to the steady firing period, T For comparison the found T w divided by T because T could vary from time to time.

Reversed pulse clamp The cell was clamped at the resting potential by setting the analogue switch high mode. The input S was set to give a depolarizing current through the cell. A short TTL-pulse switch C to low mode and the current preset at S was passed through the now unclamped cell. By adjusting t duratio of the pulse different numbers of spikes could be elicited before the pulse ended. When the clamp was switched on again, the membrane conductance was measured as previously described.

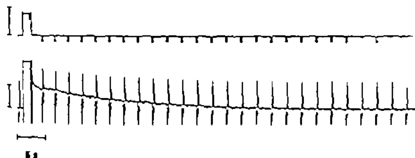
Results

A *Demonstration of the adaptation conductance*

The adaptation conductance was demonstrated in the following way The resting potential was determined and the membrane was clamped at this potential The 10 mV pulses were added, and after some time the membrane potential was changed, usually in a depolarizing direction. This change was withheld for a short time, whereafter the membrane potential was returned to the resting value. An example of such an experiment is shown in Fig. 3. The upper trace shows the membrane potential, V_m and the lower trace the transmembrane current, I_m The membrane was at the beginning of the recording clamped at the resting



Fig. 2. Onset and offset of a 20 mV pulse clamp. Recording of the membrane potential during a full compensated pulse clamp.



...ance measurements during voltage clamp. Upper trace membrane potential, lower trace ... current. 10 mV hyperpolarization every ~12 s. In the beginning of the recording 40 mV ...tion.

... (−45 mV) and 10 mV hyperpolarizing pulses of 200 ms duration were imposed ...12 s. Shortly after the start of the recording the membrane was depolarized 40 mV ...e repolarized to the resting potential after 1.8 s. During the depolarization the ...recorder was overloaded, but after the repolarization an outward current is seen, ...wly declines towards zero. The current change corresponding to the 10 mV pulses ...the depolarization larger than before the depolarization, and also this increase ...slowly with time. In Fig. 4 are shown the current in nA (circles) and the difference ...the conductance and the resting conductance (squares) as measured by the 10 mV ...units of 10^{-7} mho on a logarithmic scale as functions of the time after the end of ...larization. Both the current and the excess conductance decayed exponentially ...zero. By linear regression the time constant for the decay was found to be 16.8 s for ...nt and 17.1 for the conductance, so the current and the conductance can be taken ...with the same time constant of 17 s. This parallel decay implies that the current is ...onal to the excess conductance—the factor of proportionality being 20.3 mV in ...milar experiments with the same cell, but with different height and duration of the ...ation, yielded time constants close to 17 s and factors of proportionality close to

...ears reasonable conclusion that the slowly decaying current, triggered by the 40 ...e, was due to an increase in the membrane conductance, which declined expo... with a time constant of 17 s. The ratio of 20 mV between the current and the ...ance may correspond to the difference between the resting membrane potential and ...librium potential for the ion (ions) carrying the current. With the resting potential ...mV the equilibrium potential for the ion was then estimated to −65 mV 20 mV ...he resting potential. Different cells gave by this method equilibrium potentials ...60 and 70 mV. In some experiments the equilibrium potential was estimated ...e reversal potential for the slowly changing current. This gave similar results. ...particular cell the conductance decayed with a time constant in the interval 5–20 s, ...dently of the amplitude and duration of the depolarization, as long as the repolari... was performed to the same potential. The conductance after the depolarization ...d, however, ... both the duration and the amplitude of the depolarization. The

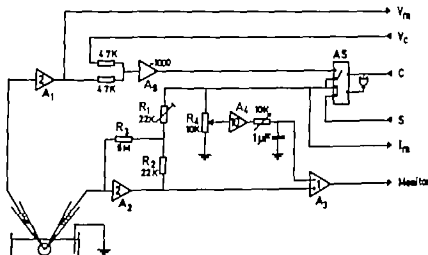


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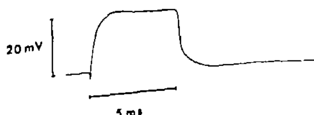


Fig. 2 Onset and offset of a mV pulse clamp Recording of membrane potential during a compensated pulse clamp.

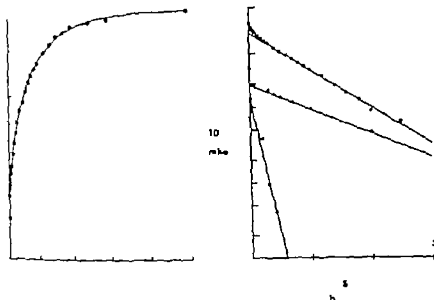


Fig. 5 Relation between the duration of 40 mV depolarization and the conductance just after the end of depolarization. Abscissa: Duration of the pulse in s, ordinate: Total conductance in units of $10^{-?}$ mho. The curve is drawn according to equation (1) in the text.

Fig. 5b Resolution of the relation in Fig. 5 in three exponentials. Abscissa: duration of the clamp (s) on different scales. Ordinate: Difference between the maximal conductance and the conductance in Fig. 5 (circles, lower scale) together with the two remaining components on different time scale (triangles and squares, upper scale).

smooth sigmoid relation between the steady state conductance and the potential with only per cent of the conductance present at the resting potential.

The relation is more curved for small conductances than for large, so the frequently used formula of the type $y = 1/(1 + e^{-x})$ does not fit the data very well. The data can, however, be approximated well by a logarithmic error function. The smooth curve in Fig. 6 is drawn according to the equation

$$g = 0.9 \cdot 10^{-?} (\mathrm{erf}(x) + 1) \tag{2}$$

with g in mho and with

$$x = \ln \frac{V + 65}{48}$$

where V is the membrane potential in units of mV

The influence of the conductance on the firing pattern of the cell

The result of a pulse clamp experiment is shown in the upper trace of Fig. 7. The cell was firing freely when a pulse of 55 mV depolarization for 60 ms was applied shortly after a spike. After the pulse the cell was silent for some time, until it resumed the steady firing. In the lower trace the firing was interrupted by two pulse clamps of the same height as

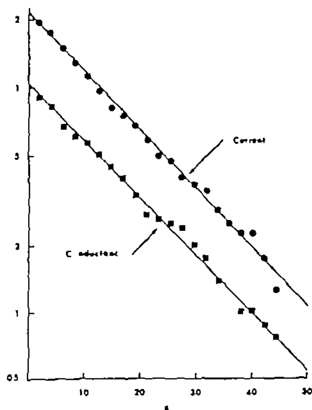


Fig. 4 Decay of membrane current and conductance after a depolarization of 40 mV [...] 2.5 s. Abscissa Time (s) after the end of the depolarization. Ordinate: Membrane current (circles) in nA and membrane conductance minus resting conductance (squares) in units of 10^{-7} mho

relation between the duration of the depolarization and the conductance just after the end of the depolarization was found by depolarizing the membrane 40 mV for durations between 20 ms and 5 s and extrapolating the conductance from the decay pattern. The conductance then rose from the resting value of 0.25 10 mho to a maximum of 1.5 10− mho (Fig. 5 a), but the rise did not follow a single exponential function. The difference between the conductance of Fig. 5 a and the maximal conductance is plotted in Fig. 5 b on a logarithmic scale (upper curve, lower time scale) With an exponential peeling off technique (Rall 1967) three exponentials could be extracted with time constants of 0.89 s, 0 14 s, and 0 012 [...] and with intercepts of 0 93 0 19 and 0.13 times 10− mho Hence, the rise of the conductance g, is for a depolarization of 40 mV given by

$$g = 1.5 - 0.13 \exp(-D/0.012) - 0.19 \exp(-D/0.14) - 0.93 \exp(-D/0.89) \quad (1)$$

in units of 10^{-7} mho and with D as the duration of the depolarization The solid curve in Fig. 5 a is drawn according to equation (1)

The relation between the steady state conductance and the membrane potential is shown in Fig. 6 In order to avoid contributions from other currents the measurements were made as the difference between the conductance just after and just before a potential change of 3 s duration to the value shown on the abscissa (resting potential −50 mV). For hyperpolarizations below −65 mV no further decrease in conductance was seen, so the [...] conductance was assumed to be zero at −65 mV measurements gave [...]

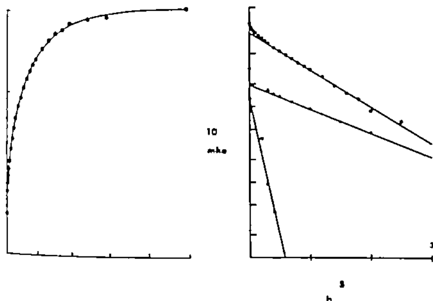


Fig. 5 Relation between the duration of 40 mV depolarization and the conductance just after the end the depolarization. Abscissa: Duration of the pulse in s, ordinate: Total conductance in units of mho. The curve drawn according to equation (1) in the text.

Fig. 5b Resolution of the relation in Fig. 5 in three exponentials. Abscissa: duration of the clamp (s) on different scales. Ordinate: Difference between the maximal conductance and the conductance in Fig. 5 (circles, lower scale) together with the two resulting components on different time scale (triangles and squares, upper scale).

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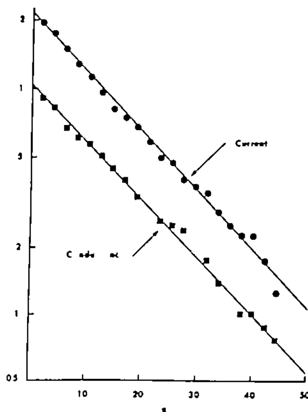


Fig. 4 Decay of membrane current and conductance after a depolarization of 40 mV 2.5 s. Abscissa: Time ( ) after the end of depolarization. Ordinate: Membrane current (circles) in nA and membrane conductance minus resting conductance (squares) in units of 10⁻ mho

relation between the duration of the depolarization and the conductance just after the end of the depolarization was found by depolarizing the membrane 40 mV for durations between 20 ms and 5 s and extrapolating the conductance from the decay pattern. The conductance then rose from the resting value of 0.25 10⁻ mho to a maximum of 1.5 10⁻ mho (Fig. 5 a) but the rise did not follow a single exponential function The difference between the conductance of Fig. 5 a and the maximal conductance is plotted in Fig. 5 b on a logarithmic scale (upper curve, lower time scale) With an exponential peeling off technique (Rig 1967) three exponentials could be extracted with time constants of 0 89 s, 0 14 s, and 0 012 and with intercepts of 0 93 0 19 and 0 13 times 10⁻ mho Hence, the rise of the conductance g, is for a depolarization of 40 mV given by

$$g = 1.5 - 0.13 \exp(-D/0.012) - 0.19 \exp(-D/0.14) - 0.93 \exp(-D/0.89) \quad (1)$$

In units of 10⁻ mho and with D as the duration of the depolarization. The solid curve Fig. 5 a is drawn according to equation (1)

The relation between the steady state conductance and the membrane potential is shown in Fig. 6 In order to avoid contributions from other currents the measurements were made as the difference between the conductance just after and just before a potential change of 3 s duration to the value shown on the abscissa (resting potential −50 mV) For hyperpolarizations below −65 mV no further decrease in conductance was seen, so the adaptation conductance was assumed to be zero at −65 mV The measurements gave

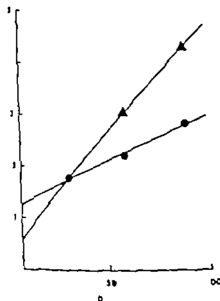


Fig. 8

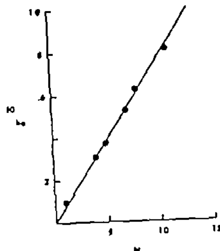


Fig. 9

Fig. 8. Relation between the total clamp duration and T_s/T for single clamps (circles) and multiple clamps of the same total duration (triangles).

Fig. 9. Relation between the number of spikes elicited during reverse pulse clamp and the increase in membrane conductance released by the spikes. Abscissa: Number N, of spikes. Ordinate: Membrane conductance just after the pulse minus the resting conductance.

ble, for the multiple pulses. The intercepts with the ordinate are 1.24 and 0.57 respectively.

For two and three pulses T_s/T is much larger than the corresponding value for single pulses lasting 60 and 90 ms. The intercept with the ordinate of 0.57 corresponds to the value of T_s/T for no pulse, or to $1 - T_s/T$ where T is the period between the spike before the pulse and the pulse. The value of T_s/T was 0.33 to 0.4 so the intercept of 0.57 corresponds fairly well to the mean value of T_s/T for no pulse. The number of pulses and their effect on T is thus proportional, and the effect therefore additive—at least for small numbers.

During a pulse clamp an increase in conductance may be triggered as described in section A. The magnitude of this increase during the different pulses, calculated from eq (1), is compared in table 1 with the prolongation of the silent period as shown in Fig. 8. It appears from the last column that the ratio between the prolongation of the silent period and the conductance is fairly constant with mean of 6.7 10⁻⁹ mho⁻ There are thus a good correlation between the conductance increase and its effect on the firing pattern, so it can be concluded that the conductance demonstrated in section A may be controlling the firing period of the cell.

To examine whether the spontaneous firing of the cell produced a conductance change as demonstrated in section A, a reverse pulse clamp was used. The result is illustrated in Fig. 9

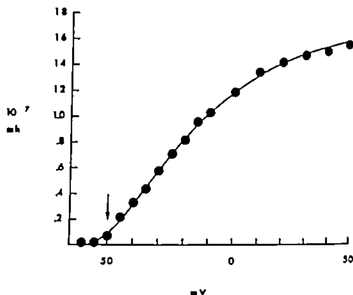


Fig. 6. Dependence of the adaptation conductance on the depolarization height. Abscissa: Depolarization from the resting potential in mV. Ordinate: The conductance just after the depolarization minus minimal value of the conductance. The resting potential is indicated by an arrow. The solid curve drawn according to eq. (2) in the text.

before, but with a duration of 30 ms and an interval of 70 ms. The silent period was m longer in the case of two pulses of 30 ms each than in the case with one pulse of 60 ms. Th phenomenon illustrates that the mechanisms responsible for the effect of the pulse on t firing pattern has a rather complicated time dependency corresponding to the ti dependency of the conductance demonstrated in section A.

The result of a series of such experiments with a depolarization of 45 mV and duratio of multiples of 30 ms is shown in Fig. 8. Each symbol represents an average of 5–15 sampl corrected as described under methods and with a S.E. of the same size as the symbols. Th abscissa is the duration of a single pulse (circles) of 30, 60 or 90 ms or the total duratio of 1, 2 or 3 pulses (triangles) of 30 ms each. The ordinate is the duration of the sile period after the pulse(s) relative to the steady firing period. The two sets of symbo form straight lines with slopes of 16.7 s for the single pulses and 40.6 s or 1.2 p

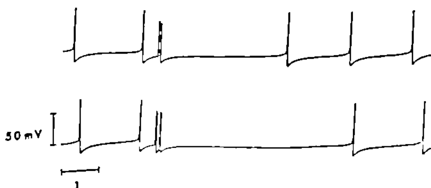


Fig. 7. Single and double pulse clamp. The upper trace shows the effect of a single pulse clamp of 60 ms. The lower trace shows the effect of two clamps of 30 ms each and with an interval of 70 ms.

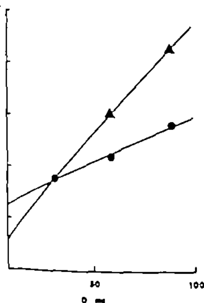


Fig. 8

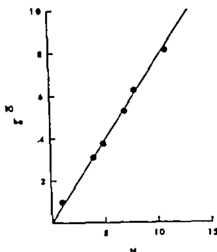


Fig. 9

Relation between the total clamp duration and T_s/T for single clamps (circles) and multiple clamps [illegible] total duration (triangles).

Relation between the number of spikes elicited during reverse pulse clamp and the increase in [illegible] conductance released by the spikes. Abscissa: Number N, of spikes. Ordinate: Membrane [illegible] just after the pulse minus the resting conductance.

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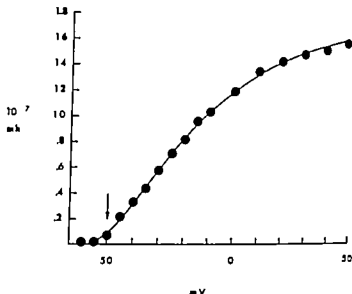


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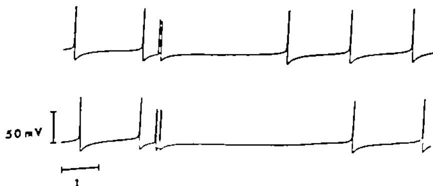


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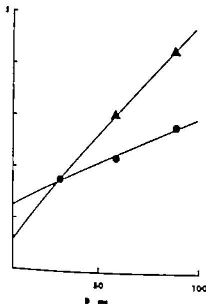


Fig. 8

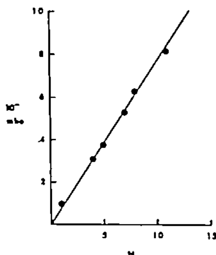


Fig. 9

Relation between the total clamp duration and T_s/T for single clamps (circles) and multiple clamps ... total duration (triangles).

Relation between the number of spikes aborted during reverse pulse clamp and the increase in ... conductance released by the spikes. Abscissa: Number, N, of spikes. Ordinate: Membrane ... just after the pulse minus the resting conductance.

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TABLE I Comparison between the relative prolongation of the silent period after pulse clamps and conductance increase calculated for similar clamps from eq (1).

Pulse duration	Relative prolongation of the silent period	Calculated conductance increase	Prolongation per mho
ms	$T_s/T - 0.57$	10⁻ mho	10' mho⁻¹
30	1.20	0.18	6.7
60	1 61	0.25	6 4
90	2.20	0.31	7 1
30	2.43	0 36	6.8
3 30	3 60	0.54	6.7

which shows that the conductance increased proportional to the number of spikes w 0 76 10⁻ mho per spike. This conductance increase per spike can be compared w the value estimated from eq (1) The half value of the depolarization for a spike from neuron was approximately 40 mV and its duration 9 ms. According to eq. (1) the ev conductance (the value of g minus the resting conductance) corresponding to 9 ms is 0 8 10^{-4} mho The effect of a single spike should therefore be of that order of magnitude.

Discussion

The main result of this investigation is that the membrane current and the clamp membrane conductance displayed almost identical decay patterns after the membrane been depolarized by 40 mV and then repolarized (Fig. 4) This strongly supports concept that the calculated conductance changes are real and makes it unlikely that current after the end of the depolarization is produced by an electrogenic pump as fo by Sokolove and Cooke (1971) for the tonic crayfish stretch receptor and by Jansen Nicolls (1973) in leech neurones. It was not possible to measure the increase of the ada tion conductance during the depolarization itself due to simultaneous changes in o conductance components. All the cells displayed a marked inactivation of the outw current (up to 90% inactivation at large depolarizations), and the adaptation current co then only be seen at small depolarizations, where the outward current rose again afte initial decline. The initial current, measured 100 ms after the beginning of a depolarizat was thus smaller than the steady state current (after 5 s) for small depolarizations, became larger than the steady state current for depolarizations larger than ?0–40 mV rise of the conductance was therefore found indirectly as shown in Fig. 5 By this met it was invariably found that the rise was much faster than the decay Normally at least time constants could be resolved one in the interval 0 7–1 0 s and another in the inte 0 1–0.3 s. The effect of the fast component in eq (1) was then seen as an apparent incr in the conductance extrapolated to zero duration of the clamp.

Thus the small time constant of 12 ms obtained for the residual fast component may be accurate, because the change in the conductance for small durations appro the limit of the method. The time constant is, however of interest, because it is of same order of magnitude as the duration of the spike, and the fast component may th fore be the main cause for the conductance increase during a single spike. This incr

from eq (1) and from the pulse clamp method found to be approximately 0.8 10^{-8} , which agrees well with the 0.65 10^{-8} mho estimated for a somewhat smaller cell by [illegible] method (Colding-Jørgensen 1977).

The pulse clamp [illegible] as a reliable method to examine the effect of the adaptation conductance [on] the firing pattern. It was, however performed with a fully adapted membrane with an [incre]ased adaptation conductance, whereas the voltage clamp was performed from a [restin]g membrane, [w]here the adaptation conductance was small. The significance of this [diffe]rence is not clear. The increased conductance before a pulse clamp might result in a [smal]ler increase during the pulse, but this effect was not observed. In the experiment with multiple pulse clamps the increase in firing period was still proportional to the number [of pul]ses, even [w]ith the tripple pulses, where the firing period became more than four times [the ste]ady state value. The effect on the firing pattern of a certain change in conductance [seem]s to be fairly constant. From table 1 the relative prolongation of the firing period is [...] 10^{7} mho^{-1} and in a previous paper (Colding-Jørgensen 1977) the conductance [increa]se of 0.65 10^{-8} mho during a single spike gave a relative increase in the firing [perio]d of 0.57 corresponding to a ratio of 9 10^{7} mho

The linear relation between T_s/T and the duration, D found in Fig. 8 is at first sight [not] in agreement with eq (1). However for durations within the interval 30–100 ms the [functi]on in eq. (1) is almost linear with a slope of 2 10^{-} mho/s. With the ratio found in [table] 1 this should correspond to a slope for the line through the circles in Fig. 8 of 13.4 s^{-1} [whic]h is close to the found 16.7 s^{-}

The results presented in section A differ from the adaptation of the molluscan neurones [descri]bed by Partridge and Stevens (1976) in several respects. They assumed that the time [consta]nt for the rise of the conductance was the same as for the decay. As seen from Fig. 5 [and eq] (1) this is definitely not the case for the impulse dependent adaptation in *HelI* The [rise] of the conductance contains time constants 3 orders of magnitude smaller than the [deca]y, and the time dependence of the conductance can therefore not be described by first [orde]r kinetics. Moreover an activation of the conductance depending on a higher order [of a] variable following first order kinetics will not apply either because it would give an [onse]t, [w]hich were slower than the offset.

[W]ith respect to the steady state value of the conductance at different membrane potentials [ther]e are also differences. Partridge and Stevens (1976) found that the conductance could [be a]pproximated by a symmetrical, sigmoid curve with a half maximum value around [...] mV and [w]hich were almost maximal at the resting potential whereas the present [findi]ngs gives an asymmetrical curve with only 5 per cent of the maximal value present [at th]e resting potential (Fig. 6). There are thus marked differences between the adaptation [of th]e two cell types. While the type presented in the previous sections definitely displays an [impuls]e dependent behaviour the cells presented by Partridge and Stevens (1976) seem [to b]e of another type, which has been called stimulus dependent (Colding-Jørgensen 1976). [Suc]h cells have also been demonstrated in *Helix* neurones (Colding-Jørgensen 1976 b).

The nature of the adaptation conductance is not clear but it has been shown that the [entr]y of calcium ions through the membrane has a marked influence on the conductance [of] the membrane, most probably the potassium conductance (Baker Hodgkin and

Ridgeway 1971 Feltz, Krnjević and Lisiewicz 1972 Meech 1972, 1974 Kostyuk, Krisht and Doroshenko 1974 Meech and Standen 1975 Vassort 1975 Heyer and Lux 197 Furthermore, the decay of membrane conductance of *Aplysia* and *Helix* after calcium injection seems to contain time constants of approximately the same magnitude the time constant for the adaptation conductance found in the present study (Meech 197 1974)

The increase in potassium conductance following a depolarization may thus be a res of a calcium influx, and the complex time dependency of the conductance may th reflect the movement of calcium ions during and after the depolarization. In this way t fast component of the conductance could be due to a rapid calcium influx passing throu the same pathway as during the normal spike.

The fundamental assumption for the impulse dependent adaptation is that each spi gives rise to a constant amount of adaptation current. This has been shown to be the ca for *Helix* neurones The current can be due to an electrogenic pump or an increased pota sium conductance as the most obvious possibilities. The present findings demonstrate th the membrane conductance increases during the adaptation and that the ion question most probably is the potassium ion, because the estimated equilibrium potenti for the adaptation current of −60 to −70 mV corresponds to the equilibrium potent for the potassium ion in snails (Connor and Stevens 1971 Neher 1971 Meech 1974 Go 1974 Meech and Standen 1975 Partridge and Stevens 1976)

In the model (Colding Jorgensen 1976 c) a simple time dependency was assumed, and t steady state conductance was assumed to be very low for potentials near the restir potential The impulse dependency was then brought foreward by assuming that t adaptation conductance only became finite during a spike and decayed towards zero b tween the spikes. The present findings disclose another type of impulse dependency Th conductance becomes finite already at small depolarizations, but during a fast potenti change—as during a spike—the fast onset of the conductance results in a rapid increase conductance, while the slow decay in the interval between the spikes is responsible f the accumulation of the conductance increase

Both types do however give rise to an almost constant amount of adaptation curre per spike, so their behaviour during repetitive firing can be assumed to be almost identic

A complete mathematical description of the kinetics of the adaptation conductance not possible on basis of the present findings. The onset of the conductance is only giv for depolarizations of 40 mV and the offset only at the resting potential. Depolarizatio to different potentials yield similar results, but further experiments are needed in ord to make it possible to give a quantitative description of the adaptation conductance

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Acta physiol. scand. 1977 101 394-403

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Angiotensin II Intratubular and Renal Capillary Pressure Regulation in the Rat Kidney

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Abstract

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Experiments were designed to test the hypothesis that angiotensin II, in its action on receptor sites of vascular smooth muscle and the proximal tubule, acts homeostatically to stabilize hydrostatic pressure in the proximal tubule, and as a consequence, maintains a constant flow of fluid to the distal tubule. Infusion at 20 nl min⁻ of Ileu⁵ angiotensin II [(Ileu⁵)-A II] (20 g ml⁻¹) into peritubular capillaries adjacent to proximal tubule was found to be accompanied by an increase in hydrostatic pressure in both the infused capillaries and the proximal tubule and by a decrease in the rate of proximal fluid reabsorption (RPFR). RPFR was derived from timed quantitative collections of tubular fluid from the last superficial proximal segments. Tubular pressure before infusion and pressures in noninfused capillaries were not related to plasma renin activity (PRA). 1 Sarcosine, 8-alanine-angiotensin II [(Sar¹ Ala⁸)-A II] is known to be a competitive antagonist to the action of angiotensin II on vascular smooth muscle and on adrenocortical zona glomerulosa cells. Infusion, at 20 nl min⁻ of (Sar¹ Ala⁸)-A II (333 ng ml⁻) into peritubular capillaries caused a decrease in hydrostatic pressure in the peritubular capillaries and adjacent proximal tubule. The most striking fall in pressure (−4 cmH$_2$O) occurred in rat with high levels of PRA. No pressure change resulted from infusion with a mixture of (Ileu⁵)-A II and (Sar¹ Ala⁸)-A II. (Sar¹ Ala⁸)-A II in the mixture blocked the inhibitory effect of (Ileu⁵)-A II on RPFR, but infusion of the angiotensin antagonist alone did not influence RPFR. It is concluded that angiotensin II is physiological regulator of hydrostatic pressure in both the proximal tubule and the peritubular capillaries.

Key words: Angiotensin II, angiotensin II antagonist glomerulotubular balance, intratubular pressure, capillary pressure, rat nephron, proximal tubule

The flow rate of sodium chloride to the distal tubule is known to influence the renin activity and the hydrostatic pressure in the glomerular capillaries of a single rat nephron (29 9 10 24 32). In addition, infusion of Ileu angiotensin [(Ileu⁵)-A II]—believed to be the circulating angiotensin II in the rat (21)—into 1st order peritubular capillaries (2) adjacent to the proximal tubule was found to elicit an increase in hydrostatic pressure in both the infused

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nd the proximal tubule and a concurrent decrease in the rate of proximal fluid
(RPFR) (28).

nts were designed to test the hypothesis that angiotensin II in its action on
s of cells in vascular smooth muscle and the proximal tubule, acts homeostatically
a constant hydrostatic pressure in the proximal tubule. Such effects will be
f angiotensin II can be displaced from its receptor sites by a competitive antago-
ould produce a decrease in hydrostatic pressure in the peritubular capillaries
ximal tubule together with an increase in RPFR. Accordingly peritubular
f the rat kidney were infused with an analogue of angiotensin II, 1-sarcosine,
giotensin II [(Sar^1 Ala^8)-A II], which has been shown to act as a competitive
o the action of angiotensin II on vascular smooth muscle in the rat (19 26) and
merulosa cells in the rat adrenal cortex (26, 30).

Methods

re made on sodium pentobarbital anaesthetized male Wistar rats weighing between 230
eparation of the animals, surgical procedures, micropuncture and analytical techniques were
described (27 28).

plasma renin activity (PRA) was achieved by infusing isotonic saline at different rates.
n rate was 2 0 ml priming infusion sustained until preparation of the kidney was complete
$^{-1}$ followed by 1 2 ml h^{-1} the minimum rate was 0 6 ml h^{-1} without any priming. The maxi-
rate with saline containing 65 mCi inulin-^{14}C-carboxylic acid (Radiochemical Centre, Amer
was used for most experiments involving tubular fluid collections, an intermediate rate, compris-
ming infusion, sustained by 1 2 ml h^{-1} throughout, was used in the remainder of these experi-
were associated with peritubular capillary infusion of (Sar^1 Ala^8)-A II. The range in PRA
ng ml^{-1} h^{-1} was achieved by the use of the maximum and the intermediate rates. This small
infusion rates is unlikely to have exerted direct measurable influence on intratubular and
apillary pressures.

measured in duplicate by radioimmunoassay (20) of blood collected from the femoral artery
the experiment. In most of the expts involving intratubular and capillary pressure measure-
ood specimens for PRA determination were obtained few minutes after making the pressure
s.

lne solutions were made up freshly each day: one containing 20 ng ml^{-1} (Ileu^5)-A II the
ng ml^{-1} or 666 ng ml^{-1} (Sar^1 Ala^8)-A II and third comprising 20 ng ml^{-1} (Ileu^5)-A II plus
(Sar^1 Ala^8)-A II. These solutions were prepared from aliquots of isotonic saline containing
(Ileu^5)-A II (Schwartz Bioresearch, Orangeburg, N Y U.S.A) and 0 01 mg ml^{-1} (Sar^1 Ala^8)-
h Pharmaceutical Co Norwich, N Y U.S.A) maintained at 20°C in glass vials. A few drops
ne green were added to 1 to 3 ml of each solution for colouration, this resulted in fall in
5 to 6 31. Solutions were infused into the peritubular capillaries using the Sonnenberg Deetjen
t at the rate of 20 nl min^{-1} throughout (28). At this infusion rate, 20 ng ml^{-1} (Ileu^5)-A II
relatively high physiological dose (28)

e technique (27 28) was used to measure hydrostatic pressure in 1st order peritubular capil-
d with (Sar^1 Ala^8)-A II and in proximal tubules associated with similarly infused capillaries.
additional measurements were made in noninfused capillaries or in proximal tubules before
sion. T examine the ability of (Sar^1 Ala^8)-A II to block the effect of (Ileu^5)-A II, these
were repeated infusing the peritubular capillaries with (Ileu^5)-A II, or the mixture of (Ileu^5)-A II
la^8)-A II, or with isotonic saline alone.

of (Sar^1 Ala^8)-A II on rat proximal nephron function was studied to determine whether the
ffect of angiotensin II on hydrostatic pressure in the proximal tubule is mediated wholly or
anges in RPFR. Peritubular capillaries were infused alternately with isotonic saline and either
A II or the mixture of (Ileu^5)-A II plus (Sar^1 Ala^8)-A II. RPFR, nephron GFR and hydrostatic
re measured in the adjacent tubules

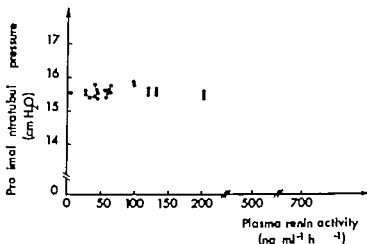


Fig. 1 Relationship between hydrostatic pressure in the proximal tubule and plasma renin activity (PRA). Variation in PRA was achieved by using different rates of i.v. infusion of isotonic saline

Timed quantitative fluid collections were made from the last superficial proximal segments. The tubular fluid collection time was approximately 130 s throughout. The mean ratio of the oil block insertion time to the tubular fluid collection time was between 0.09 and 0 10 for all sample groups. For all expts. involving fluid collections, the mean capillary infusion time was between 7 and 8 min Kidney GFR and the femoral artery blood pressure were also recorded.

Nephron GFR was calculated from the tubular fluid to plasma inulin ratio, TF/P inulin, and the volume of tubular fluid collected per minute, V using the following expression nephron GFR = TF/P inulin. The absolute rate of tubular fluid reabsorption to the site of puncture was calculated as follows: nephron GFR − V̇ For statistical evaluation the two-tailed sign test was employed with a 5 % level of significance (25).

Results

Hydrostatic pressure

a) *Intratubular pressure* (Fig. 1 and 2) Pressures in proximal tubules before infusion varied very little despite large differences in PRA from one rat to another

Infusion of the peritubular capillaries with (Sar Ala)-A II caused a significant decrease in hydrostatic pressure in the adjacent proximal tubules ($p < 0.05$) (28 measurements in l rats) The magnitude of this decrease varied the most striking fall in pressure occurred in rats with relatively high levels of PRA suggesting that the angiotensin II antagonist inhibited endogenous angiotensin II

The large variations in PRA in association with a constant intratubular pressure, coupled with the changes in hydrostatic pressure which could be induced by the infusion of (Sar Ala)-A II strongly suggest that angiotensin II is continuously exerting a regulatory effect on pressure in the proximal tubule.

With (Ileu⁸)-A II alone as the infusate, hydrostatic pressure in the proximal tubules was significantly increased ($p < 0.05$) (8 measurements in 6 rats), but no significant pressure change resulted from infusion of the (Ileu)-A II plus (Sar Ala)-A II mixture (17 measurements in 10 rats).

b) *Capillary pressure* (Table I) Changes in capillary pressure were similar to those observed intratubularly Infusion with (Sar Ala)-A II caused a significant decrease ($p < 0.05$) in peritubular capillary pressure again the most striking reduction was observed in rats with relatively high PRA

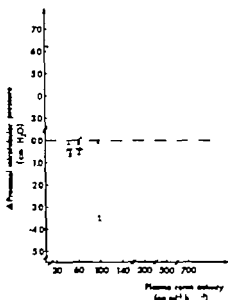


Fig. 2 Effect on hydrostatic pressure in the proximal tubule of peritubular capillary infusion (20 nl min^{-1}) with 20 ng ml^{-1} (Ileu^5)-A II (squares); 333 ng ml^{-1} (Sar^1 Ala^8)-A II (triangles); 20 ng ml^{-1} (Ileu^5)-A II plus 333 ng ml^{-1} (Sar^1 Ala^8)-A II mixture (circles) in rats with different plasma renin activities (PRA). Changes in intratubular pressure are plotted as a function of PRA. Variation in PRA was achieved by using different rates of i.v. infusion of isotonic saline.

Table 1 Effect on hydrostatic pressure in the peritubular capillaries of infusion (20 nl min^{-1}) with (Ileu^5)-A II (20 ng ml^{-1}), (Sar^1 Ala^8)-A II (333 ng ml^{-1}), (Ileu^5)-A II (20 ng ml^{-1}) plus (Sar^1 Ala^8)-A II (333 ng ml^{-1}) mixture; isotonic saline. The mean value of each expt. is given. The number of measurements made and used to generate each mean value is given in parentheses.

Expt. No.	Capillary pressure noninfused (cmH_2O)	Capillary pressure infused (cmH_2O)	Blood pressure (mmHg)	PRA (ng ml^{-1} h^{-1})
Infusate [Ileu^5]-A II				
	16 6 (1)	23 8 (1)	114	—
	16 0 (2)	21.5 (1)	130	—
	17 9 (2)	23 6 (3)	128	—
	17 6 (2)	22 7 (3)	124	62
Infusate [Sar^1 Ala^8]-A II				
	15 4 (1)	12.4 (3)	126	119
	15 8 (2)	13 3 (2)	125	97
	16 0 (2)	13 4 (1)	116	45
	13 1 (3)	13.4 (1)	115	59
	17.5 (3)	15 9 (3)	116	57
	17 0 (2)	15 8 (2)	167	42
	17 5 (1)	16 2 (1)	120	66
Infusate [Ileu^5]-A II + [Sar^1 Ala^8]-A II				
	16 0 (1)	16.4 (1)	116	45
	15 2 (1)	15 0 (1)	114	59
	17 4 (2)	16 2 (2)	116	62
Infusate Isotonic saline				
	16 6 (1)	17.2 (2)	114	—
	15 9 (2)	16.7 (2)	117	—

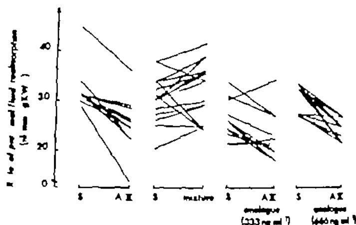


Fig. 3 Effect on the rate of proximal fluid reabsorption (RPFR) of peritubular capillary infusion (? min) with 20 ng ml^{-1} (Ileu[5])-A II 20 ng ml^{-1} (Ileu[5])-A II plus 333 ng ml^{-1} (Sar[1] Ala[8])-A II with 333 ng ml^{-1} (Sar[1] Ala)-A II 666 ng ml^{-1} (Sar[1] Ala[8])-A II In each rat, tubular fluid collections made *alternately* from different tubules associated with peritubular capillary infusion of either isotonic saline (S) or one of the experimental solutions. The lines connect the individual values derived from each rat.

Infusion with (Ileu[5])-A II produced an increase in capillary pressure, but no change resulted from infusion of the (Ileu[5])-A II plus (Sar[1] Ala)-A II *mixture*.

Isotonic saline infusion caused a small increase in capillary pressure, slightly greater th that previously reported for intratubular pressure (28).

Arterial blood pressure was not related to PRA level.

Proximal nephron function (Fig. 3 4 and 5 values obtained previously with infusion (Ileu[5])-A II are included for comparison)

Infusion with (Ileu[5])-A II has been shown to cause a significant ($p < 0.05$) decrease in RPF (28).

Infusion with the mixture of (Ileu[5])-A II plus (Sar Ala[8])-A II failed to cause consiste changes in RPFR ($p > 0.05$), suggesting that the angiotensin II analogue blocked the obser effect of (Ileu[5])-A II on RPFR

No significant change in RPFR was caused by infusion with 333 ng ml (Sar[1] Ala)-A I but when the concentration was increased to 666 ng ml a decrease in RPFR was co sistently observed

Nephron GFR is unaffected by the infusion of (Ileu[5])-A II (28) Infusion with 333 ng ml (Sar Ala[8])-A II was similarly ineffective ($p > 0.05$), but nephron GFR increased slight although not significantly ($p > 0.05$) when the mixture of (Ileu)-A II plus (Sar Ala[8])-A was infused. Infusion of 666 ng ml (Sar Ala)-A II consistently resulted in a decrease i nephron GFR, despite a simultaneous substantial decrease in intratubular pressure.

Indices of whole kidney function and PRA level in experiments related to proximal nephron function

Mean kidney GFR and mean arterial blood pressure measured in association with infusio of each type of experimental solution or with infusion of isotonic saline are given in Table I Neither of the paired differences departed significantly from zero ($p > 0.05$)

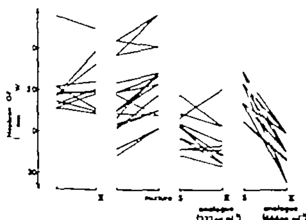


Fig. 4 Effect on nephron GFR of peritubular capillary infusion (20 nl min⁻¹) with: 20 ng ml⁻¹ (Ileu⁵)-A II, 20 ng ml⁻¹ (Ileu⁵)-A II plus 333 ng ml⁻¹ (Sar¹ Ala⁸)-A II mixture; 333 ng ml⁻¹ (Sar¹ Ala⁸)-A II; 666 ng ml⁻¹ (Sar¹ Ala⁸)-A II. In each rat, tubular fluid collections were made alternately from different tubules associated with peritubular capillary infusion of either isotonic saline (S) or one of the experimental solutions. The lines connect the individual values derived from each rat.

PRA was measured in 5 of the 9 expts. associated with (Ileu⁵)-A II infusion, 6 of the 7 with the mixture of (Ileu⁵)-A II plus (Sar¹ Ala⁸)-A II, 3 of the 6 with 333 ng ml (Sar Ala⁸)-A II, and in both expts. with 666 g ml (Sar¹ Ala)-A II mean values for PRA (and range) respectively were 46 (5–83), 88 (60–130), 116 (64–202), and 158 (151–166) ng ml h⁻¹

Discussion

The experimental data provide strong evidence that the hydrostatic pressure in the peritubular capillaries is maintained by the vascular action of angiotensin II and further indicate that angiotensin II plays a homeostatic role in the regulation of hydrostatic pressure in the proximal tubule. A constant flow rate to the distal tubule may result from stabilizing hydrostatic pressure in the proximal tubule.

The present results agree with earlier observations (5) that infusion of (Sar Ala⁸)-A II into the renal artery of the sodium depleted dog decreased renal vascular resistance and increased renal blood flow: infusion did not alter these functions in the normal dog. Renal sodium excretion did not change during (Sar Ala⁸)-A II infusion. In the rabbit, similar observations have been made (18).

In some preliminary expts. (Steven, unpublished), alternate infusion of 20 ng ml and 80 ng ml⁻¹ (Ileu⁵)-A II into the same peritubular capillary resulted in a clearly visible reduction in capillary diameter in association with the more concentrated solution. The action of angiotensin II in stabilizing hydrostatic pressure in the capillaries could therefore be mediated partly by inducing changes in capillary diameter. This does not preclude the possibility that stability results mainly from changes in postcapillary resistance.

Evaluation of the way in which angiotensin II stabilizes hydrostatic pressure in the proximal tubule on of the variables influencing this pressure, i.e. an

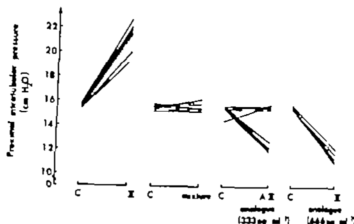


Fig. 5 Effect on hydrostatic pressure in the proximal tubule of peritubular capillary infusion (20 nl ml with 20 ng ml⁻ (Ileu⁸)-A II 20 ng ml⁻¹ (Ileu⁸)-A II plus 333 ng ml⁻ (Sar¹ Ala⁸)-A II mixture 333 ng t (Sar¹ Ala⁸)-A II 666 ng ml⁻¹ (Sar Ala⁸)-A II Measurements were made in each tubule before (contr C) and during peritubular capillary infusion with one of the experimental solutions. The lines connect individual values derived from each rat. Results are given only from expts. involving tubular fluid col tions.

analysis of nephron hydrodynamics. Since decreases in hydrostatic pressure along proximal and distal tubules are very small (7 8 15 16, 31) there are effectively onl resistances to fluid flow in each nephron glomerular resistance loop of Henle resistan and collecting duct resistance. For the total number of nephrons within the kidney each these 3 resistances is connected in parallel. For analytical purposes, it is convenient consider the equivalent nephron" a hypothetical single nephron which has the sa hydrodynamic characteristics as the entire population of nephrons in the kidney For

TABLE II Indices of whole kidney functio I the expts related to proximal nephron function.

Infusate	GFR (ml min gKW⁻)		Mean arterial pressure (mmHg)	
	E[a]	S[b]	E[a]	S[b]
[Ileu⁸]-A II 20 ng ml⁻¹ (range)	1.37 (1 16–1 62)	1.51 (1.31–1 80)	118 (94–131)	118 (100–131)
[Ileu⁸]-A II 20 ng ml⁻ plus [Sar¹ Ala⁸]-A II 333 ng ml⁻ (range)	1 47 (1 13–1 78)	1.52 (1 25–1.82)	111 (95–119)	112 (107 120)
[Sar¹ Ala⁸]-A II 333 ng ml⁻ (range)	1.37 (1 14–1 60)	1 45 (1 28–1 65)	121 (108–128)	119 (107 126)
[Sar¹ Ala⁸]-A II 666 ng ml⁻¹ (range)	1.22 (0.83–1.54)	1.33 (0.95–1.50)	129 (116–137)	133 (118–142)

Experimental infusate. [b] Isotonic saline infusate.
Neither of the paired differences departed significantly from zero (p

quivalent nephron, the pressure head, which may be termed the glomerular propulsive ressure (13), that drives fluid against the nephron resistance is equal to the difference etween the mean glomerular capillary pressure, less the mean glomerular oncotic pressure, nd the renal pelvic pressure.

Equivalent glomerular and loop of Henle resistances are found by dividing the respective esistances of the single nephron by the total number of nephrons in the kidney approximately 30 000 in the rat (11). Collecting duct resistance seems to be located mainly in the erminal collecting duct (17 22). Assuming that collecting duct resistance is exclusively erminal, an approximate value for equivalent collecting duct resistance is given by dividing he resistance of the single collecting duct by the number of terminal collecting ducts in the idney approximately 20 in the rat (11).

These calculations involve some simplification, in that all nephrons are assumed to function in an identical manner but they provide an appropriate first approximation since ecent measurements in the inner medulla of the rat kidney have shown that the hydrostatic ressure in the loop of Henle of the juxtamedullary nephron is similar to that of the super icial nephron (22). From experimental measurements in the hydropenic rat (1 2, 3 6, 7 , 12, 14, 3 31), the following values may be derived equivalent glomerular resistance 1 10^{-4} mmHg nl min, loop of Henle resistance 15 10^{-4} mmHg nl min, collecting duct resistance 700 10^{-4} mmHg nl min. The hydrodynamic resistance of the glomerular membrane is accordingly very small compared with tubular resistance, of which the great majority is encountered at the distal extreme of the tubular system.

Increasing the glomerular capillary pressure increases GFR and the urine flow rate, but the percentage of additional filtered fluid reabsorbed will be smaller than the percentage of fluid reabsorbed previously This observation taken together with the distribution of hydrodynamic resistances calculated above will gi e the equivalent nephron the following definite characteristics, always assuming that tubular resistance is constant. GFR may be considerably changed by small alterations in RPFR but is influenced only to a small extent by changes in glomerular propulsive pressure. Conversely hydrostatic pressure in the proximal tubule will be relatively insensitive to changes in RPFR but will be sensitive to the most modest perturbations in glomerular propulsive pressure. Although constant tubular resistance has been assumed in the foregoing calculations, the inverse changes in tubular resistance which are known (17) to occur in response to variations in intratubular pressure will stabilize the hydrostatic pressure in the proximal tubule and further decrease the small effect of alterations in RPFR on that pressure.

Infusion of peritubular capillaries with 20 ng ml^{-1} (Ileu^5)-A II resulted in a significant decrease in RPFR. Infusion with the mixture of 20 ng ml^{-1} (Ileu)-A II plus 333 ng ml (Sar Ala^8)-A II did not cause any consistent change in RPFR, which suggests that (Sar^1 Ala^8)-A II blocked the effect of (Ileu^5)-A II observed previously

When 333 ng ml (Sar Ala^8)-A II alone was similarly infused, no consistent change was found in RPFR despite the relatively high PRA levels. This finding is difficult to reconcile with the observation that (Sar Ala^8)-A II blocked both the vascular and tubular effects of (Ileu^5)-A II. We can, as yet, offer no explanation for this discrepancy Nevertheless, it follows from the foregoing outline of nephron hydrodynamic characteristics, that the influence of

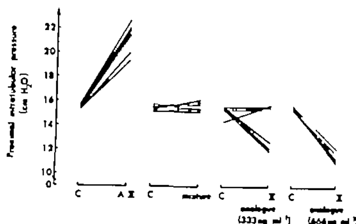


Fig. 5 Effect on hydrostatic pressure in the proximal tubule of peritubular capillary infusion (20 nl min⁻¹) with 20 ng ml⁻ (Ileu⁸)-A II 20 ng ml⁻ (Ileu⁸)-A II plus 333 ng ml⁻ (Sar¹ Ala⁸)-A II mixture 333 ng ml⁻¹ (Sar¹ Ala⁸)-A II 666 ng ml⁻¹ (Sar¹ Ala⁸)-A II Measurements were made in each tubule before (control C) and during peritubular capillary infusion with one of the experimental solutions. The lines connect the individual values derived from each rat. Results are given only from expts. involving tubular fluid collections.

analysis of nephron hydrodynamics. Since decreases in hydrostatic pressure along the proximal and distal tubules are very small (7 8, 15 16, 31), there are effectively only 3 resistances to fluid flow in each nephron glomerular resistance loop of Henle resistance and collecting duct resistance. For the total number of nephrons within the kidney each of these 3 resistances is connected in parallel For analytical purposes, it is convenient to consider the "equivalent nephron" a hypothetical single nephron which has the same hydrodynamic characteristics as the entire population of nephrons in the kidney For the

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[a] Experimental infusate. [b] Isotonic saline infusate.
Neither of the paired differences departed significantly from zero (p > 0 0

angiotensin II or indeed of any substance, on proximal intratubular pressure cannot be effected by changes in RPFR but must involve changes in either the glomerular propulsive pressure or the tubular resistance. The effect of angiotensin II on intratubular pressure is probably mediated by variations in the ultrafiltration pressure in the glomerular capillaries since it appears unlikely that the intratubular pressure could be regulated by changes in tubular resistance that is itself very sensitive to tubular pressure (P Koch Jensen and K. Steven, unpublished) An augmentation in glomerular capillary pressure increases tubular pressure and so increases the flow rate to the distal tubule. In addition, the increase in tubular pressure will result in a decrease in tubular resistance, causing a further increase in the flow rate to the distal tubule. An efficient regulatory mechanism for the glomerular capillary pressure will therefore stabilize the flow rate to the distal tubule and prevent over-elevation of the flow rate in relation to the reabsorptive capacity of the distal tubule and collecting duct.

Infusion of peritubular capillaries with 666 ng ml (Sar Ala[8])-A II was performed in two experiments. In both a large decrease was observed in intratubular pressure, associated with a substantial decrease in nephron GFR and with a marked *reduction* in RPFR. Despite the small number of observations, it is apparent that (Sar Ala[8])-A II in this concentration at the infusion rate employed, exerted an agonistic, angiotensin II-like effect on RPFR while simultaneously antagonising the vascular action of angiotensin II

The present expts. clearly indicate that angiotensin II is a physiological regulator of the hydrostatic pressure in the proximal tubule and the peritubular capillaries of the rat nephron. The effect of angiotensin II on intratubular pressure is probably mediated by variations in capillary pressure in the glomerular capillaries

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In Vivo Turnover Rate of Acetylcholine in Rat Brain Parts at Elevated Steady-State Concentration of Plasma Choline

By

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Abstract

ECKERNÄS, S Å L. SAHLSTRÖM and S M AQUILONIUS. *In vivo turnover rate of acetylcholine in rat brain parts at elevated steady state concentration of plasma choline* Acta physiol scand. 1977 *101* 404–410

Cortical and striatal turnover rates of acetylchol ne TR_{ACh} were estimated by applying steady-state tracer kinetics in rats killed by microwave irradiation following constant i.v infusion of ^{3}H-Ch. I control rats TR_{ACh} was 3 6 nmol g min in the cortex and 23 8 nmol g^{-} min^{-1} in the striatum. When steady state plasma concentrations of Ch were increased from 17 to 140 µmol l by a 15-min infusion of unlabelled Ch the correspond ng TR_{ACh} were 3 6 nmol g^{-1} min^{-} and 1 4 nmol g^{-1} min^{-} respectively These result indicate that increased plasma level of Ch re not accompanied by increased synthesis of brain ACh

Key words. Acetylcholine, turnover rate, plasma choline, rat brain parts

Several regulatory mechanisms for the synthesis of acetylcholine (ACh) in the cholinergic neurons have been suggested (for ref see Browning 1976, Baker 1976, Haubrich 1976). It seems probable that the availability of choline (Ch) in the nerve terminal is the rate-limiting step in ACh synthesis (Eisenstadt and Schwartz 1975 Simon *et al* 1976). Recently it has been suggested by several investigators that an increase in plasma Ch concentration can increase the availability of the ACh precursor and thus enhance the rate of ACh synthesis This suggestion is based on the observation that brain ACh levels are elevated by the systemic administration of a large dose of Ch (Cohen and Wurtman 1975 1976, Haubrich *et al* 1974, 1975 Wurtman and Fernstrom 1976) However a direct agonistic effect of Ch cannot be ruled out, since no attempts have been made to determine the brain turnover rate of ACh at an increased plasma Ch level

In the present paper we have applied the principles of steady state tracer kinetics (Zilversmit 1960) following a constant i v infusion of tritiated Ch (H-Ch) for the calculation of brain ACh turnover rates in rats with different steady state concentrations of Ch in plasma.

Methods

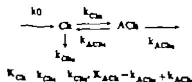
General. Male Sprague-Dawley albino rats weighing 220–240 g were used. The animals were housed for one week with food *ad libitum* before the expts., which took place at the same time of the day. The rats were killed by focusing a beam of microwaves on the head for 1.7 s (5 kW, 2 450 MHz, Metabolstat, Gerling Moore Inc., USA). Brain temperature measured 5 mm below the bregma 10 s after irradiation was about 95 C. These conditions are known to stop *post mortem* changes in brain Ch and ACh (Schmidt 1976, Stavinoha *et al.* 1973, Nordberg 1977). Immediately after sacrifice 100 μl of blood was drawn by means of heart puncture and the parietal cortex and striatum were dissected out. The tissue samples were homogenized in 5 ml ice-cold 15% 0.1 M formic acid in acetone and further processed as described earlier (Eckernäs and Aquilonius 1977).

Determination of Ch and ACh. The brain Ch and ACh concentration and plasma Ch concentration were analyzed by a radio-chemical procedure (Eckernäs and Aquilonius 1977 a, b).

Measurement of radioactive Ch and ACh. In an aliquot of the tissue extracts ACh and Ch were separated by means of high voltage paper electrophoresis (Aquilonius and Eckernäs 1976). After localization with iodine vapour the spots were cut out and eluted in 4 ml of methanol in a scintillation vial. After adding 10 ml of scintillation cocktail (Permablend III Packard 77 mg ml^{-1} toluene), the vials were kept in darkness for 12 h before the radioactivity was measured by liquid scintillation counting (Isocap-300, Searle Nuclear Chicago Division).

Estimation of ACh turnover rate. A tracer dose (47 nmol kg^{-1} min^{-1}) of H-Ch (10.6 Ci mmol^{-1} Amersham, England) was infused (1 ml kg^{-1} min^{-1} 0.5 mCi kg^{-1} min^{-1}) for 2 or 3 min, using a motor syringe. During the infusion the animals were in place in the holder of the microwave equipment and the rats were killed immediately after the infusion.

In some expts. the steady-state concentration of plasma Ch was increased by means of a 15-min infusion of unlabelled Ch (0.25 ml kg^{-1} min^{-1} 15 μmol kg^{-1} min^{-1}) before the infusion of H-Ch. The two infusions were then continued simultaneously for a further 2 or 3 min. Brain ACh turnover rates were calculated by measuring the change with time of the specific radioactivity of brain Ch (S_{Ch}) and ACh (S_{ACh}). The following kinetic model was assumed:



$$K_{Ch} \quad k_{Ch} \quad k_{Ch}; \; K_{ACh} - k_{ACh} + k_{ACh}$$

K_{Ch} and K_{ACh} denote the fractional rate constants of brain Ch and ACh compartments, respectively, *i.e.* the fractions of the compartments that are renewed per unit of time (min^{-1}). The turnover rate of ACh (TR_{ACh} nmol g^{-1} min^{-1}) is then calculated from the following formula

$$TR_{ACh} \quad K_{ACh} \text{ (ACh concentration)} \tag{1}$$

Under the assumption that *steady-state conditions exist* and that the change in brain S_{Ch} and S_{ACh} with time can be mathematically handled as if brain Ch and ACh are each in a homogeneous compartment where random processes prevail, the following equation describes the change of S_{ACh} with time (Zilversmit 1960)

$$\frac{dS_{ACh}}{dt} = K_{ACh}(S_{Ch} \quad S_{ACh}) \tag{2}$$

The equation can be solved for K_{ACh} as follows. The function f( ) is assumed to be any function which describes the change of S_{Ch} with time (S_{Ch} f(t)). f(t) is inserted in equation 2 to yield.

$$\frac{dS_{ACh}}{dt} = K_{ACh}(f(t) - S_{ACh}) \tag{3}$$

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In Vivo Turnover Rate of Acetylcholine in Rat Brain Parts at Elevated Steady-State Concentration of Plasma Choline

By

SVEN ÅKE ECKERNÄS, LENA SAHLSTRÖM and STEN-MAGNUS AQUILONIUS

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Abstract

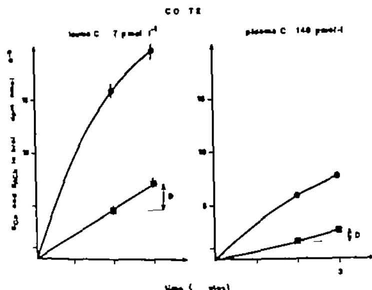
ECKERNÄS, S Å., L. SAHLSTRÖM and S M AQUILONIUS. *In vivo turnover rate of acetylcholine in rat brain parts at elevated steady state concentration of plasma choline* Acta physiol scand 1977 *101* 404–410

Cortical and striatal turnover rates of acetylcholine TR_{ACh} were estimated by applying steady-state tracer kinetics in rats killed by microwave irradiation following a constant i.v. infusion of H-Ch. In control rats TR_{ACh} was 3 6 nmol g⁻ min the cortex and 23 mol g⁻ min the striatum. When steady state plasma concentrations of Ch were increased from 17 to 140 μmol l⁻ by 15-min infusion of unlabelled Ch the corresponding TR_{ACh} were 3 6 nmol g⁻ min and 21 4 nmol g^{-1} min^{-1} respectively. These results indicate that increased plasma levels of Ch are not accompanied by increased synthesis of brain ACh.

Key words: Acetylcholine, turnover rate, plasma choline, rat brain parts

Several regulatory mechanisms for the synthesis of acetylcholine (ACh) in the cholinergic neurons have been suggested (for ref see Browning 1976, Baker 1976, Haubrich 1976). It seems probable that the availability of choline (Ch) in the nerve terminal is the rate-limiting step in ACh synthesis (Eisenstadt and Schwartz 1975 Simon *et al* 1976). Recently it has been suggested by several investigators that an increase in plasma Ch concentration can increase the availability of the ACh precursor and thus enhance the rate of ACh synthesis. This suggestion is based on the observation that brain ACh levels are elevated by the systemic administration of a large dose of Ch (Cohen and Wurtman 1975 1976 Haubrich *et al* 1974, 1975 Wurtman and Fernstrom 1976) However a direct agonistic effect of Ch cannot be ruled out, since no attempts have been made to determine the brain turnover rate of ACh at an increased plasma Ch level

In the present paper we have applied the principles of steady-state tracer kinetics (Zilversmit 1960) following a constant i v infusion of tritiated Ch (H-Ch) for the calculation of brain ACh turnover rates in rats with different steady state concentrations of Ch in plasma.

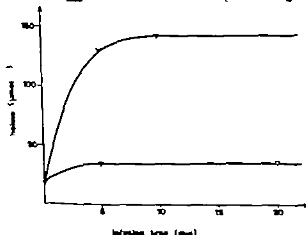


Specific radioactivity of Ch and ACh in the cortex during an I infusion of ³H-Ch in rats infused with saline or unlabelled Ch. ●—● S_{Ch} ■—■ S_{ACh} K_{ACh} = D/(shaded area).

...m the ACh concentration was slightly increased (84 nmol g^{-1}), while the cortical ...eared to be unchanged.

...nges in S_{Ch} and S_{ACh}. Fig. 1 and 2 shows the alteration in S_{ACh} and S_{ACh} in the brain ...s during a constant i. infusion of H-Ch at different steady-state levels of plasma Ch.

...nover rate of brain ACh. The calculated TR ch of saline treated animals was 23.8 g^{-1} min^{-1} in the striatum and 3.6 nmol g^{-} min^{-1} in the cortex (Table I). Elevation ...sma Ch levels seemed to have no effect on cortical TR ch (3 6 nmol g^{-} min^{-1}), ...a slight decrease in TR_{ACh} was found in the striatum (21 4 nmol g^{-1} min^{-1} Table I).



The change in plasma Ch concentration in carotid blood of unanaesthetized rats during constant i ...on of unlabelled Ch. ▼—▼ 15 µmol kg^{-1} min^{-1}; ▽—▽ 2 5 µmol kg^{-1} min^{-1}

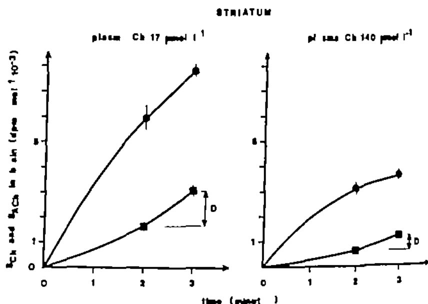


Fig. 1 Specific radioactivity of Ch and ACh in the striatum during an i.v. infusion of ³H-Ch in rats infused for 15 min with saline or unlabelled Ch. ●—● S_{Ch} ■—■ S_{ACh} $K_{ACh} = D$/(shaded area).

which can be integrated between two time points t_1 and t_2.

$$S_{ACh_2} - S_{ACh_1} = K_{ACh}\left(\int_{t_1}^{t_2} f(t)\,dt - \int_{t_1}^{t_2} S_{ACh}\,dt\right) \quad (4)$$

The difference at the left is equal to D and the quantity between the square brackets is equal to the shaded area in Fig. 1 and 2. Thus

$$K_{ACh} = \frac{D}{\text{shaded area}} \quad (5)$$

By approximating the changes in S_{Ch} and S_{ACh} between 2 and 3 min to straight lines, K_{ACh} can easily be calculated.

In some rats the brain blood volume was estimated with ³H-dextran (Aquilonius and Eckernäs 1976) and it was concluded that Ch treatment did not alter the remaining blood volume in brain tissue.

Results

Elevation of plasma Ch. In Fig. 3 the change in plasma Ch concentration during a constant i.v. infusion of unlabelled Ch (15 µmol kg⁻¹ min⁻¹ and 2.5 µmol kg⁻¹ min⁻¹) to anesthetized rats is shown. The steady state level is reached within 10 min (Fig. 3) and seems to be linearly related to the infusion rate (Fig. 4). In the unanesthetized rats, used in the turnover calculations, the final steady-state level of plasma Ch following the infusion of unlabelled Ch was also about 140 µmol l⁻¹.

Brain Ch and ACh concentrations. The results are presented in Table I. In rats infused with saline the striatal Ch and ACh concentrations were 33 ± 1.2 and 76 ± 2.9 nmol g⁻¹ respectively while the corresponding values in the cortex were 23 ± 2.5 and 16 ± 1.0 nmol g⁻¹.

When plasma levels of Ch were increased to 140 µmol l⁻¹ by means of a constant i.v. infusion of unlabelled Ch for 15 min, significantly increased concentrations of brain Ch were detected both in the striatum (53 nmol g⁻¹) and in the cortex (59 nmol g⁻¹). In the

was calculated from D_i(shaded area) in Fig. 1 and 2. The theoretical error introduced by this simplification is negligible in consideration of the experimental error inherent in each value (Fig. 1 and 2). The estimated TR_{ACh} values in control rats are almost identical with those recently reported by Racagni *et al.* (1974).

One problem with the estimation of TR_{ACh} following administration of Ch is that the system must be at steady-state if the equations described under Methods shall be valid. To fulfil this criterion the plasma Ch concentrations had to be elevated by means of a constant i.v. infusion of unlabelled Ch prior to an infusion of ^{3}H-Ch. As shown in Fig. 3 steady-state levels of plasma Ch are rapidly achieved during an i.v. infusion and these levels seemed to be linearly related to the infusion rate (Fig. 4), which is in agreement with the result of Freeman *et al.* (1975). Thus, after a 15-min infusion of unlabelled Ch, the plasma Ch must fulfil the steady-state criterion. From Fig. 4 it is obvious that the infusion of ^{3}H-Ch (47 nmol $kg^{-1}min^{-1}$) does not significantly perturb the steady-state conditions of Ch.

The mean elevated plasma Ch level (~140 µmol l^{-1}) is of the same magnitude as the peak values shown to be paralleled by increased brain ACh concentrations in the studies by Haubrich *et al.* (1975) using i.p. administration of Ch. In the present study the Ch concentrations were significantly increased both in the cortex and in the striatum following the infusion of Ch, while ACh was increased slightly (11 %) in the striatum only (Table I). This is in accordance with the idea that an increased concentration of plasma Ch will be paralleled by increased steady-state concentrations of brain Ch and ACh (Cohen and Wurtman 1975, Haubrich *et al.* 1974, 1975). However, in rats with plasma Ch concentrations elevated to 140 µmol l^{-1} no definite changes in the regional ACh turnover rates were observed. These findings thus contradict the suggestion that administration of Ch will enhance the neuronal synthesis of ACh in the brain.

It is well known that centrally active muscarinic agonists (*e.g.* oxotremorine) increase the ACh concentration in the brain but decrease TR_{ACh} (Nordberg 1977, Trabucchi *et al.* 1975). Thus, at present the most probable explanation for the slight but significant increase in striatal ACh concentration and the minute reductions of striatal TR_{ACh} would seem to be a direct agonistic effect of Ch itself in some receptor-close compartment, resulting in feedback inhibition of neuronal activity, or that the increase in ACh concentration is localized at a "non-releasable pool" of ACh.

However the present results do not necessarily rule out the possibility that cholinergic function will be enhanced by the administration of Ch under pathological conditions (Aquilonius and Eckernäs 1975, 1977).

We are indebted to Mrs Inger Falk and Miss Lisbeth Lehtipalo for technical assistance. Our thanks are due to Professor B. Holmstedt for kindly allowing us to use the microwave equipment at the Department of Toxicology at Karolinska Institutet, Stockholm.

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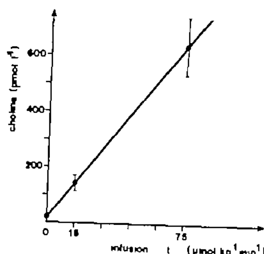


Fig. 4. Steady-state levels of plasma Ch as a functi[on] of the rate of infusion of unlabelled Ch.

Discussion

The aim of this study was to evaluate the proposals recently put forward by several autho[rs] (Cohen and Wurtman 1975, 1976, Haubrich *et al.* 1974, 1975, Wurtman and Fernstrom 197[6]) that increased concentrations of plasma Ch stimulate the synthesis of brain ACh.

Under steady state conditions an enhanced transmitter synthesis must be balanced by a[n] increased transmitter outflow and thus result in an increased turnover rate of the transmitte[r] (Zilversmit 1960).

The validity of the equations formulated by Zilversmit and co-workers (for review se[e] Zilversmit 1960) for calculation of the brain ACh turnover rate would seem questionable since the nature and number of various "pools" of Ch (Aquilonius and Eckernäs 197[6]) and ACh (Jenden *et al.* 1974, Nordberg 1977) are unknown components. It is also known at least *in vitro* that newly synthesized ACh is preferentially released (Collier 1969, Potte[r] 1970, Molenaar and Polak 1976). However, it has been clearly shown by Cheney *et al.* (1975) that these equations do seem to be valid for the calculation of ACh turnover rate, at least during the first 20 min, following the administration of radioactive precursor.

In the present study a tracer dose of ^{3}H-Ch was infused i.v. in rats and the S_{Ch} and S_{ACh} of brain tissue were measured after a 2 and 3 min infusion. The change of brain S_{Ch} and S_{ACh} with time was approximated to straight lines in the time interval 2–3 min and K_{ACh} …

TABLE I. Cholinergic parameters in brain regions of the rat at different steady-state concentrations of plasma Ch.

Brain part and steady-state concentration of plasma Ch	Ch (nmol g⁻¹) $\bar{m} \pm$ S.E.	ACh (nmol g⁻¹) $\bar{m} \pm$ S.E.	Fractional rat constant K_{ACh} (min⁻¹)	Turnover rate TR_{ACh} (nmol g⁻¹ min⁻¹)
Cortex (plasma Ch 17 µmol l⁻¹, n = 18)	23 ± 2.4	16 ± 1.6	0.22	3.6
Cortex (plasma Ch 140 µmol l⁻¹, n = 15)	59 ± 3.7*	14 ± 1.7	0.26	3.6
Striatum (plasma Ch 17 µmol l⁻¹, n = 23)	33 ± 1.22	76 ± [illegible]9	0.31	23.8
Striatum (plasma Ch 140 µmol l⁻¹, n = 22)	53 ± [illegible]0*	84 ± 3.0	0.26	21.4

$p < 0.02$, $p < 0.001$ (Student's t test).

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Acta physiol. scand. 1977 101 411–417

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Effect of the Preceding Day's Energy Intake on the Total Energy Cost of Light Exercise

By

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Abstract

GARBY L. and O. LAMMERT *Effect of the preceding day energy intake on the total energy cost of light exercise* Acta physiol. scand. 1977 *101* 411–417

The effect of variation in the preceding day's dietary energy intake–4, 10 and 18 MJ–on the oxygen consumption and respiratory quotient at rest and at work before and after test meal (4 MJ) was investigated. 7 normal male subjects were studied at 3 different experimental situations. During the first day the subjects had an energy intake of 10 MJ and normal activity. The following morning measurements of $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were performed at rest and at work on bicycle ergometer (34 W) before and after the test meal. The same measurements were performed on the following day but the dietary intake between the two sets of measurements was then either 6, 0 or 14 MJ. No significant effect was found of the preceding day dietary energy intake on the oxygen consumption or on the energy transformed during rest, during exercise before and after the test meal. The results do no support the conclusions of previously published studies, proposing significant feed-back mechanisms tending to control the body energy content by regulation of the energy expenditure through chemical or mechanical uncoupling.

The ways in which human beings control their energy balance are poorly understood. It has been suggested that control takes place by regulation of both energy intake and energy expenditure, but neither the relative importance nor the mechanisms of these processes have been adequately defined. The literature up to 1973 has been reviewed by GARROW (1974), see also SIMS (1976).

Miller Mumford and Stock (1967) and Apfelbaum, Bostsarron and Lacatis (1971) have published results that can be interpreted in terms of changes in efficiency during work when energy intake has been markedly changed for several weeks. Recently Miller and Wise (1975) have presented results, which indicate that the cost of light arm exercise varies with the energy content, at normal composition, of the preceding day's food. This finding suggests the existence of a feedback system operating on the efficiency of transformation of chemical energy also after a short disturbance in energy intake. The data of Miller and Wise do not allow a definite interpretation of the quantitative role of the suggested phenomenon, but indicate that a significant part of the surplus energy intake on one day

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From the Department of Physiology and the Department of Physical Education, Odense University Denmark

Effect of the Preceding Day's Energy Intake on the Total Energy Cost of Light Exercise

By

LARS GARBY and OLE LAMMERT

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Abstract

GARBY L. and O. LAMMERT *Effect of the preceding day's energy intake on the total energy cost of light exercise* Acta physiol. scand. 1977 *101* 411–417

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can be dissipated during the next day and that quite a considerable part of an acute deficit can be recovered by increased efficiency of utilization of the next day's food intake. If substantiated and found to be valid for other activities as well these findings have far reaching implications for our understanding of how the energy balance is regulated.

The present work was undertaken in order to obtain additional information on the significance of the phenomenon described by Miller and Wise. We have measured the oxygen consumption and carbon dioxide elimination during light bicycle exercise (36 W) before and after a test meal on different days preceded by a day in which the food energy intake was varied between 4 and 18 MJ. The results show that under these conditions the variation of the total cost of this type of exercise with the preceding day's energy intake is either absent or so small as to be of questionable significance in the control system.

Materials and methods

The observations were performed on 7 apparently healthy male subjects (average age = 59±3 years, weight = 78.0±6.9 kg, height = 18 ±5.6 cm).

Experimental design. This is illustrated in Fig. 1 and was as follows. On the first day of study the subjects performed their ordinary work and other activities with the exception that all their meals were prepared in the laboratory according to standard procedure and with a fixed composition and amount so that it contained 10 MJ in total (see below). On the second day of study the subjects came to the laboratory in the morning after having fasted for 12 14 h. They rested under standard conditions for [illegible] min whereupon the resting oxygen consumption and carbon dioxide elimination were measured during 15 min. Immediately thereafter the subjects performed 36 W for 30 min on bicycle ergometer (see below) and the respiratory gas exchange was measured during the last 15 min of the period. A test meal containing 4 MJ (see below) was then given and the subjects rested horizontally for 30 min after which the same work and measurements were performed as before the test meal. The remaining part of the day was spent with ordinary activities but the food taken was restricted to that prepared in the laboratory so that an additional energy intake with a fixed composition of 0, 6 or 14 MJ was ensured. On the third day of the expt., the schedule of day was repeated with the exception that the food intake after the measurements was free. Thus, measurements of oxygen consumption and carbon dioxide elimination were performed on each subject at rest, during exercise before the test meal and during exercise after the test meal on 3 different occasions, each consisting of 2 consecutive days of which the first followed day with an intake of 10 MJ and the second followed a day with an energy intake of 4, 10 or 18 MJ.

Work performance and respiratory gas exchange

The oxygen consumption and carbon dioxide elimination were measured using low-resistance modif[illegible] Otis-McKerrow valve and the Douglas bag method. The bags were preinflated by the subjects immediately before the actual collection of expired air in order to ensure that the dead space in the bags had a[illegible] and oxygen and carbon dioxide concentrations close to those resulting from the actual measurements. Three bags were collected for each experimental period. The volume was measured in calibrated 1901 spirometer and the oxygen and carbon dioxide concentrations were measured a Servomex OA 184 oxygen analyser and a Beckman LB1 carbon dioxide analyser respectively. Both instruments were calibrated using several gas mixtures with oxygen and carbon dioxide concentrations measured by the micro-Scholander technique (P. F. Scholander 1967). The expiratory air was dried in silicagel filter before admitted to the analysers.

From every third Douglas bag, 5 ml samples were drawn and the concentrations of oxygen and carbon dioxide were also measured using the micro-Scholander technique. This procedure was performed in order to check the stability of the instruments used for gas analysis. The mean value and the standard deviation of the 64 differences (instrument minus micro-Scholander) in oxygen concentration were 0.03 ±0.05% (absolute) and the corresponding values for carbon dioxide concentration were 0.03±0.03 (absolute).

The Monark bicycle ergometer was calibrated before and several times during the test periods

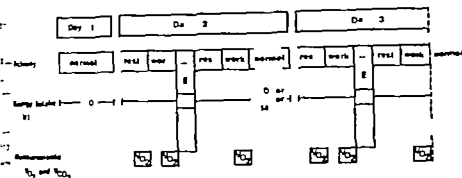


Diagram showing experiments design

Fig 1 The experimental design repeated once week over 3 eeks period Day 1 The subject ate all the standard meals at the laboratory (10 MJ), no measurements ere performed Day 2 and 3 $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were measured at the end of the rest period and work period before the test meal (4 MJ), and at the end of the work period after the meal Between these two days measurements all meals ere eaten at the laboratory The energy contained in these meals were either 0–6 or 14 MJ

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In 6 of the subjects, all expts. ere performed ith one eek intervals on the same days in order to obtain similar patterns of daily physical activity In one subject, all expts were performed consecutively The results from this subject did not differ from those found in the other 6 subjects. All the subjects followed the same dietary schedule first the 10–10 MJ expt then the 10–4 MJ and finally the 10–18 MJ expt.

Composition and energy content of food

Among the food items commonly used in Denmark, suitable number was selected which could easily be handled and weighed 3 standard meals—breakfast, lunch and dinner—were prepared by commonly used cooking methods ith the exception that no item was fried ith fat or oil Each item was eighed and the composition and energy content of each meal were calculated according to Helms (1973). The daily intake of 18 MJ was achieved by increasing the amount of each item in lunch and dinner by factor of 2.5

The energy content of one day meals, from each of the 4, 10 and 18 MJ days, was determined by

Table 1 Composition and energy content of meals

Meal	Composition, of total energy as				Energy content, MJ	
	Carbohydrate	Fat	Protein	Alcohol	Calculated	Measured
Breakfast (test meal)	47 8	36 7	14 8	0	4 2	4 24
Lunch	35.2	40 7	17 7	7.0	2.9	
Dinner	42 6	37 3	14 1	6 0	3 4	
Total 10 MJ	42 6	37.9	15.3	3 9	10 5	10.2
Total 18 MJ	41.1	38.3	15 5	5 0	18.2	17 2
Total 4 MJ	47 8	36 7	14 8	0	4 2	4 24

can be dissipated during the next day and that quite a considerable part of an acute deficit can be recovered by increased efficiency of utilization of the next day's food intake. If substantiated and found to be valid for other activities as well these findings have far reaching implications for our understanding of how the energy balance is regulated.

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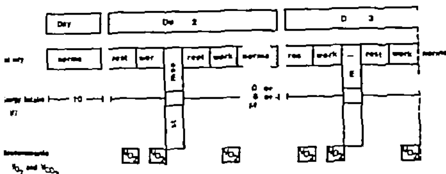


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In 6 of the subjects, all expts. were performed with one week intervals on the same days in order to obtain similar patterns of daily physical activity. In one subject, all expts. were performed consecutively. The results from that subject did not differ from those found in the other 6 subjects. All the subjects followed the same dietary schedule: first the 10–10 MJ expt., then the 10–4 MJ and finally the 10–18 MJ expt.

Composition and energy content of food

Among the food items commonly used in Denmark, a suitable number was selected which could easily be divided and weighed. 3 standard meals—breakfast, lunch and dinner—were prepared by commonly used cooking methods with the exception that no item was fried with fat or oil. Each item was weighed and the composition and energy content of each meal were calculated according to Helms (1973). The daily intake of 18 MJ was achieved by increasing the amount of each item in lunch and dinner by a factor of 2.25.

The energy content of one day's meals, from each of the 4, 10 and 18 MJ days, was determined by

TABLE 1 Composition and energy content of meals

Meal	Composition, % of total energy as				Energy content, MJ	
	Carbohydrate	Fat	Protein	Alcohol	Calculated	Measured
Breakfast (test meal)	47.8	36.7	14.8	0	4.2	4.24
Lunch	35.2	40.7	17.7	7.0	2.9	
Dinner	42.6	37.3	14.1	6.0	3.4	
Total 10 MJ	42.6	37.9	15.3	3.9	10.5	10.2
Total 18 MJ	41.1	38.3	15.5	5.0	18.2	17.2
Total 4 MJ	47.8	36.7	14.8	0	4.2	4.24

can be dissipated during the next day and that quite a considerable part of an deficit can be recovered by increased efficiency of utilization of the next day's food If substantiated and found to be valid for other activities as well, these findings h reaching implications for our understanding of how the energy balance is regulat

The present work was undertaken in order to obtain additional information significance of the phenomenon described by Miller and Wise We have meas oxygen consumption and carbon dioxide elimination during light bicycle exercise before and after a test meal on different days preceded by a day in which the foo intake was varied between 4 and 18 MJ The results show that under these c the variation of the total cost of this type of exercise with the preceding day's ener is either absent or so small as to be of questionable significance in the control sy

Materials and methods

The observations were performed on 7 apparently healthy male subjects (erage age 25.9 weight = 78 0 ± 6.9 kg, height = 182 ± 5 6 cm).

Experimental design. This is illustrated F g. 1 and was as follows On the first day subjects performed their ordinary work and other act vities with the exception that all their prepared in the laborat ry according t a standard procedure and w th a fixed compositio so that it co tained 10 MJ in total (see below) O the second day of study the subject laboratory in the morn ng after having fasted for 12 14 h They rested under standard cond min whereupon the restl g oxygen consumption and carbon dioxide el mination were me 15 min. Immediately thereafter the subjects perf rmed 36 W for 30 min on bicycle ergomet and the respiratory gas exchange was measured during the last 15 min of the period A test m 4 MJ (see below) was then gi en and the subjects rested horizontally for 30 min after work and measurements were performed as before the test meal. The remaining part of the with ordinary activities but the food taken was restricted to that prepared i the laborat add tional energy intak with a fixed composition of 0 6 or 14 MJ was ensured. On the t expt., the schedule of day 2 was repeated with the exception that the food take after the was free Thus, measurements of oxygen consumption nd carbon dioxide elimination ere each subject at rest, during exercise bef re the test meal and during exercise after the different occasions, each consisti g of consecutive days of which the f rst followed intake of 10 MJ and the second followed day with energy intake of 4, 10 or 18 MJ

Work performance and respiratory gas change

The oxygen consumption and carbon dioxide elimination were measured using a low resist Otis-McKerrow valve and the Douglas bag method The bags were preinflated by the subject before the actual collection of expired air n order t ensure that the dead space n the bags and oxygen and carbon dioxide concentrations close t those result ng from the actual Three bags were collected f each experimental period. The olume as measured in a spirometer and the oxygen and carbon dioxide concentrations were measured a Ser oxygen analyser and a Beckma LB1 carbo dioxide analyser respectively Both ins calibrated using several gas m tures with o ygen and carbo d oxide concentrations me micro-Scholander technique (P F Scholander 1967). The expiratory air was dried n a bcar admitted to the analysers.

From every third Douglas bag, 5 ml samples were draw and the concentrations carbon dioxide were also measured using the micro-Scholander technique. This procedure in order to check the stability of the instruments used for gas analysis. The mean value an deviation of the 64 differences (instrument micro-Scholander) o ygen concentratio ±0 05% (absolute) and the corresponding values for carbon dioxide concentration were (absolute).

The Monark bicycle ergometer was cal brated before and several t mes during the

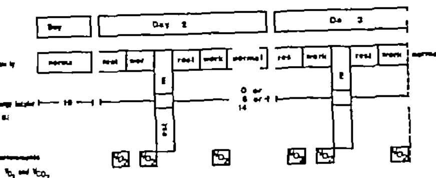


Diagram showing experimental design

Fig. 1. The experimental design repeated once a week over a 3 weeks period. Day 1: The subject ate all the prescribed meals at the laboratory (10 MJ), no measurements were performed. Day 2 and 3: $\dot{V}o_2$ and $\dot{V}co_2$ were measured at the end of the rest period and work period before the test meal (4 MJ), and at the end of the work period after the meal. Between these two days measurements all meals were eaten at the laboratory. The energy consumed in these meals were either 0–6 or 14 MJ.

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The energy content of one day's meals, from each of the 4, 10 and 18 MJ days, was determined by

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	Carbohydrate	Fat	Protein	Alcohol	Calculated	Measured
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Table III Respiratory exchange ratio during exercise before and after the test meal on two consecutive days following an energy intake of 10 and 4 MJ and of 10 and 18 MJ

Subjects	Before the test meal						After the test meal					
	10	4	Δ	10	18	Δ	10	4	Δ	10	18	Δ
P.J.	0.746	0.711	0.035	0.762	0.720	−0.042	0.801	0.784	0.017	0.845	0.830	−0.015
S.E.D.	0.796	0.777	0.019	0.849	0.858	+0.007	0.854	0.837	−0.017	0.863	0.881	+0.018
G.H.R.	0.800	0.738	0.062	0.733	0.807	+0.064	0.848	0.804	−0.044	0.821	0.819	−0.002
A.R.	0.813	0.790	−0.023	0.842	0.846	+0.004	0.853	0.836	−0.017	0.876	0.890	+0.024
L.P.	0.827	0.796	0.051	0.785	0.810	+0.025	0.862	0.823	−0.039	0.835	0.859	−0.024
S.B.	0.798	0.746	0.052	0.811	0.814	+0.003	0.827	0.766	−0.061	0.843	0.832	−0.011
O.J.	0.810	0.741	−0.069	0.790	0.782	−0.008	0.848	0.829	−0.019	0.815	0.812	−0.003
Mean	0.799	0.754	0.044	0.796	0.805	+0.008	0.842	0.811	−0.031	0.842	0.846	+0.005
S.D.	0.026	0.028	0.019	0.042	0.045	0.032	0.021	0.027	0.018	0.022	0.031	0.017
S.E.	0.010	0.010	0.007	0.016	0.017	0.012	0.008	0.010	0.007	0.008	0.01	0.007

differences are also small, being 0 and −18 ml O min^{-1} for the effects before the test meal and 7 and 6 ml O_2 min^{-1} after the test meal. None of the mean differences are statistically significant. Both before and after the test meal the oxygen consumption decreased on the day preceded by an intake of 18 MJ as compared with that on the day preceded by an intake of 4 MJ the values being −27 ± 20 and −22 ± 12 ml O min^{-1} respectively.

The effect of the test meal on the oxygen consumption during exercise is also shown in Table II. The mean values before and after the test meal differ by about 50 ml O min with no dependence on the previous day's intake.

The mean oxygen consumption during rest on two consecutive days following 10 and 4 MJ energy intake was 2 ± 6 ml O min^{-1} lower on the day following 4 MJ. On two consecutive days following 10 and 18 MJ it was 2 ± 8 ml O min^{-1} (n 7) higher on the day following 18 MJ.

The respiratory exchange ratio observed during exercise are shown in Table III. The differences due to the test meal are about 0.05 and highly significant. The differences due to the previous day's intake, 10 MJ versus 4 MJ are highly significant, the latter being smaller by 0.04 and 0.03 before and after the test meal respectively. No significant differences were observed when comparing the values followed by a dietary intake of 10 and 18 MJ.

Discussion

Miller and Wise (1975) measured the cost of light arm exercise before and after a test meal containing 3.9 MJ at 3 levels of the preceding day's energy intake: 4, 10 and 17 MJ. In 4 subjects the cost before the test meal was found to be independent of the previous day's intake and was 2.8, 2.4 and 2.6 kJ min^{-1} (mean values), respectively. However after the test meal the mean values were 1.8, 2.3 and 3.0 kJ min respectively and this effect of the preceding day's intake was statistically significant. Thus, the cost of the postprandial exercise decreased by 1.0 kJ min^{-1} or 36 %, when the previous day intake was low and it increased by 0.4 kJ min^{-1} or 15%, when the previous day's intake was high.

TABLE II Oxygen consumption (ml O_2 STPD min^{-1}) during exercise before and after the test meal on two consecutive days following an energy intake of 10 and 4 MJ and of 10 and 18 MJ respectively

Subject	Before the test meal							After the test meal						
	10 MJ	4 MJ	Δ (4–10)	10 MJ	18 MJ	Δ (18–10)	Δ (18–4)	10 MJ	4 MJ	Δ (4–10)	10 MJ	18 MJ	Δ (18–10)	(18–4)
P J	852	830	−22	839	894	+55	+64	947	931	−16	950	947	−3	16
S. E. D	747	774	+27	756	680	−76	−94	786	776	−10	767	806	+39	+30
G H R.	896	869	−27	907	879	−28	+10	994	864	−30	924	934	+10	70
A. R.	773	776	+3	730	739	+9	−37	783	853	+70	762	806	+44	47
L. P	810	827	+17	780	772	−8	−55	853	852	−1	832	797	−35	55
S. B	750	785	+35	749	723	−26	−62	796	827	+31	835	806	−29	21
O J	931	899	−32	933	882	−51	−17	945	948	+3	974	903	−71	45
Mean	823	823	0	813	796	−18	−27	872	878	+7	863	857	−6	22
S D	73	48	27	81	88	42	52	89	70	34	86	68	42	33
S.E.	27	18	10	31	33	16	20	34	26	13	33	26	16	1[illegible]

bomb calorimetry (IKA C 400, Janke und Kunkel AG Staufen i Br Germany) after homogenisation in a Waring blender The heat released in the calorimeter was corrected by subtracting 5.04 KJ for each gram of protein in the mixture.

The composition and energy content of the meals are shown in Table I

Results

The values for oxygen uptake and respiratory exchange ratio (R) measured during rest and during work at 36 W were found to be very similar to those usually found in young apparently healthy male subjects and the average net efficiency of the work was 17.9 Considering the relatively low work intensity this is a normal value, *cf* Gaesser and Brooks (1975) Also the heart rate and the ventilation/oxygen consumption ratio were normal for the conditions used During exercise, 60 min after the test meal, the heart rate was, on an average, 12 beats per minute higher than during work before the test meal This is in agreement with what has been found elsewhere, *cf* Dagenais, Oriol and McGregor (1966

The results of measurements of oxygen consumption on 2 consecutive days were as follows (1) at rest 249 ml min ±29 ml min on day two and 243 ml min ±28 ml min on day three, (2) during exercise before the test meal 822 ml min ±75 ml min on day two and 870 ml min ±110 ml min on day three, (3) during exercise after the test meal 890 ml min ±103 ml min on day two and 912 ml min ±118 ml min on day three. The sum of the variations due to methodological error and day-to-day variation was calculated for each of the conditions of rest exercise before the test meal and exercise after the test meal and found to be 4.5 6 1 and 6.9% (coefficient of variation) respectively

The effect of the preceding day's dietary energy intake on the oxygen consumption during exercise is shown in Table II where the actual value on one day is compared to the corresponding value on the previous day the latter preceded by a dietary intake of 10 MJ The differences are rather small and range between −76 and +70 ml O min The mea

have no explanation for this discrepancy Only the type of work, arm exercise or cle exercise, was different in the two studies. A feedback mechanism of the general suggested by several authors, Apfelbaum *et al* (1971) and Miller and Wise (1975), not seem to be operative under the conditions studied by us, i.e acute perturbations e order of 6–8 MJ of the input. Although such perturbations are large with respect to "normal" input, they are very small in relation to the body energy stores, i.e about MJ if the signals for control of energy expenditure are related to the latter a difference only 1 may be too small to activate the mechanism.

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The findings of Miller and Wise suggest the existence of an important feed-back loop in the regulation of the total energy balance of the body Thus, out of a surplus of 7 MJ, added to the total body energy content during one day of overeating, not less than 0.4 MJ of the dietary energy of the next day s intake could be dissipated by chemical or mechanical "uncoupling" if one assumes that light exercise of the type studied by Miller and Wise takes up 16 h of the day Furthermore, not less than 1 MJ of a 6 MJ deficit, incurred by one day s energy-deficient diet, could be recovered by an increased efficiency of utilization of the next day s food energy

The results obtained in the present work can be discussed in terms of the mechanism suggested by Miller and Wise. It is, however necessary first to consider how the measured values of oxygen consumption can be converted into values for the energy transformed during the different periods of the study The facts that the workload was light and that $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ calculations were made from analyses of gas collected during the last 15 minutes of 30 min working periods make it reasonable to believe that the ventilatory exchange quotient represents a true value for whole body RQ Furthermore, the observed mean value of the pulmonary ventilation rate at work of about 22 l min supports this belief The energy transformed in association with the consumption of one liter of oxygen depends on the pathways involved in the oxidation and, since different steady state respiratory exchange ratios were observed in the various periods, it is necessary to use different factors for this conversion.

Benedict and Carpenter (1909 1910) measured the heat production and respiratory gas exchange in adult subjects performing little or no muscular activity (300–500 ml oxygen per minute) and on a normal diet. A plot of the heat produced per liter of oxygen consumed *vs* the respiratory quotient shows a rather considerable scatter but a mean increase of the former of about 1 4 kJ per liter or about 6 %, can be estimated for the RQ interval between 0 75 and 0 85 This value is considerably higher than that calculated on the assumption of complete stochiometric combustion of normal food *i.e* about 0 6 kJ per liter The estimate would not be expected to vary significantly with moderate variations in protein intake and breakdown, *cf* Schulz (1975), and we assume that it is also relatively independent of moderate variations in physical activity

The present work shows that a change in the energy content of the preceding day s meals (4 MJ *vs* 18 MJ) was associated with a mean increase of the respiratory exchange ratio of 0 051 and 0 035 (see Table III) before and after the test meal respectively The mean decrease in the oxygen consumption under these conditions was 3 3 (823 − 796 27 ml O_2 min) and 2.4% (878 − 857 − 21 ml O_2 min), respectively (Table II). When these values are converted to changes in energy transformation and corrected for the difference in RQ they become 0.2 and 0.3% respectively If when correction for differences in RQ is made a value of 0 6 kJ per liter is used instead of the value of 1 4 kJ per liter (see above), the energy transformation rate becomes slightly lower after 18 MJ than after 4 MJ These numbers are associated with the same relative standard errors as that of the mean decrease in oxygen consumption, *i.e* 2.4 and 1.3 %, respectively Thus, the energy transformation at the same work intensity is practically independent of the preceding day s energy intake.

The present results are thus at variance with those presented by Miller and Wise (1975).

We have no explanation for this discrepancy. Only the type of work, arm exercise *vs.* bicycle exercise, was different in the two studies. A feedback mechanism of the general type suggested by several authors, Apfelbaum *et al.* (1971) and Miller and Wise (1975), does not seem to be operative under the conditions studied by us, I [illegible] acute perturbations of the order of 6–8 MJ of the input. Although such perturbations are large with respect to the "normal" input, they are very small in relation to the body energy stores, I [illegible] about 600 MJ. If the signals for control of energy expenditure are related to the latter a difference of only 1 % may be too small to activate the mechanism.

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glomerular population. Observations on glomeruli in deeper cortical layers are therefore highly desirable.

The aim of the present study was to investigate whether micropuncture and pressure measurements could be made on deep cortical structure after surgical exposure. Preliminary experiments with a chronical preparation were unsuccessful. A sagittal cut through the cortex could be kept open by a thin teflon sheet, but at reinvestigation after 1 to 7 days the cut surface was too densely covered with adherent fibrin and/or granulation tissue to permit identification of any cortical structures. At the suggestion of H. R. Ulfendahl acute exposure was attempted, and turned out to be technically feasible.

A preliminary report of this study was presented at the II European Colloquium on Renal Physiology (Aukland, Tønder and Ness, 1977).

Methods

Experiments were performed on Wistar and Sprague Dawley rats of either sex with body weight 200–300 g. Both strains [illegible] obtained from Møllegaards SPF Avlslaboratorium, Copenhagen. The animals were [illegible] standard rat pellets (Vestlandske Fetlsildfabrikk, Bergen), giving [illegible] sodium intake of approximately 2 mmol/ day. Usually food—but not water—was withdrawn in the afternoon the day before the expts. The Sprague Dawley rats and one group of Wistar rats were studied in Inactin (Promonta, Hamburg) anesthesia, given as [illegible] single intraperitoneal injection of 120 mg/kg BW. A second group of Wistar rats was anesthetized with Mebumal (pentobarbital), using an initial i.p. injection of 40 mg/kg, and additional i.v. doses of 3–5 mg/kg if needed. The rats were tracheotomized and placed on [illegible] heated operating table, the rectal temperature being controlled at 37.0°–37.5°C. For blood sampling and for recording of arterial blood pressure (P[illegible]) [illegible] polyethylene tube (PE50) was placed in the [illegible] artery or the lower [illegible] abdominal aorta via [illegible] femoral artery. A femoral vein catheter was inserted for [illegible] injections.

No fluid was given during the expts. except for occasional flushing of the catheters with isotonic saline, usually well below 2 ml in each expt. Varying amounts of saline used for flushing the kidney may have been absorbed from the peritoneum. However, the urine appeared concentrated, and urine flow was low ([illegible] 5 µl/min) [illegible] measured.

Preparation of the kidney

The left kidney was exposed through [illegible] subcostal incision and the ureter, renal artery and vein were [illegible]. While some renal nerve strands were severed during dissection, no attempt was made at complete denervation. The kidney was placed in [illegible] lucite cup [illegible] its dorsal aspect facing upward, and [illegible] by cotton moistened in saline. The ureter was cannulated in some expts. When the ureter was left intact, the maintenance of peristaltic waves [illegible] checked.

Using [illegible] sharp scalpel [illegible] 1–2 mm thick, lens-shaped slice with [illegible] diameter of about 5 mm [illegible] cut off from the dorsal aspect of the kidney (Fig. 1 A). For shortness, this procedure and the cortical defect produced by [illegible] will hereafter be referred to as corticotomy. The immediate profuse bleeding stopped spontaneously in the course of 1–3 min. Blood was removed by repeated flushing [illegible] isotonic saline and suction by micropipettes. The kidney was then covered [illegible] warm mineral oil. If not drained off "tubular fluid" would accumulate at the corticotomy under the mineral oil. [illegible] order to establish the origin of this fluid, [illegible] 0.9 [illegible] NaCl was infused [illegible] at [illegible] constant rate of 15 µl/min in 4 expts. Arterial blood, urine and fluid from the corticotomy [illegible] collected simultaneously and [illegible] concentrations determined [illegible] liquid scintillation counter. Possible reflux from the pelvis into the corticotomy [illegible] tested by puncturing the renal pelvis and injecting 0.2 ml Evans blue solution.

Maintenance of flow at the exposed glomeruli was tested by injecting Lissamine green [illegible] and watching its appearance in the microscope.

Micropuncture was performed [illegible] glass pipettes with sharpened tip of 2 to 5 µm diameter, filled with [illegible] M NaCl colored [illegible] Evans blue. Neutralization of the solution with $NaHCO_3$ seemed to prevent precipitation of Evans blue. The micropipette was mounted on [illegible] Leitz-Wetzlar micromanipulator and the punctures were performed under [illegible] Wild M5 stereomicroscope with [illegible] magnification of 75

Acta physiol. scand. 1977 101 418–427

From the Institute of Physiology University of Bergen, Norway

Capillary Pressure in Deep and Superficial Glomeruli of the Rat Kidney

By

K. AUKLAND K. HEYERAAS TØNDER and G. NÆSS

Received 25 April 1977

Abstract

AUKLAND K K. HEYERAAS TØNDER and G NÆSS. *Capillary pressure in deep and superficial glomeruli of the rat kidney* Acta physiol scand. 1977 *101* 418–427

A new technique has been developed to make deep renal cortical structures in rats accessible for micropuncture: The left kidney is dissected free and immobilized in a lucite cup. A lense-shaped slice, 1 mm thick and about 5 5 mm wide, is cut off from the dorsal aspect of the kidney Blood oozing from the cut surface is removed by flushing with saline and suction by microsponges. The bleeding stops in 1 3 min and causes none or only transient fall arterial pressure (P_A) Up to 40 glomeruli become visible and remain circulated for several hours, as shown by injection of dye or silicone rubber Glomerular capillary pressures (P_G), measured with servocontrolled counter pressure (Wiederhielm), showed no consistent change with time and no correlation to P_A Average $P_G \pm$ S.E. in mmHg (number of glomeruli in parentheses) were Wistar rats (WR), Inactin anesthesia, 57.8 ± 1.4 (41), Mebumal anesthesia, 58.1 ± 1.3 (13). Sprague Dawley rats, Inactin, 58.1 ± 1.7 (14). In WR, P_G was lower in deep than in midcortical glomeruli < 0.4 mm below kidney surface, 57.9 ± 1.8 (20) 0.5–0.9 mm 60.5 ± 1.5 (20) and > 1.0 mm 53.8 ± 2.5 (13) Pressure in Bowman's capsule, 11.2 ± 0.6 (30). The observed P_G is higher than previously reported on the Munich mutant strain of WR, and suggests that glomerular filtration equilibrium is not reached.

Key words. Bowman's capsule, glomerular capillary pressure, glomerular filtration, kidney circulation, renal hemodynamics

While previous indirect estimates of glomerular capillary pressure (P_G) ranged from 60 to 90 mmHg, direct micropuncture of superficial glomeruli in a mutant Wistar rat (Munich strain) has given values of about 45 mmHg (Brenner Troy and Daugharty 1971 Blantz *et al* 1972) As a consequence of the latter estimate, the net filtration pressure is low and filtration equilibrium is reached before the end of the average glomerular capillary and the glomerular filtration rate will be strongly dependent on glomerular blood flow (Deen, Robertson and Brenner 1972, 1974) However recent P_G measurements of 62.6 mmHg in superficial glomeruli which may be occasionally observed on the ventral aspect of the kidney in Sprague Dawley rats (Källskog *et al* 1975 a) have seriously questioned the validity of the Munich Wistar strain as a representative model. In any case, it is not known whether the accessible superficial glomeruli are representative for the whole or a great part of the

glomerular population. Observations on glomeruli in deeper cortical layers are therefore highly desirable.

The aim of the present study was to investigate whether micropuncture and pressure measurements could be made on deep cortical structure after surgical exposure. Preliminary experiments with a chronical preparation were unsuccessful. A sagittal cut through the cortex could be kept open by a thin teflon sheet, but at reinvestigation after 1 to 7 days the cut surface was too densely covered with adherent fibrin and/or granulation tissue to permit identification of any cortical structures. At the suggestion of H. R. Ulfendahl acute exposure was attempted, and turned out to be technically feasible.

A preliminary report of this study was presented at the II European Colloquium on Renal Physiology (Aukland, Tønder and Naess, 1977).

Methods

Experiments were performed on Wistar and Sprague Dawley rats of either sex with body weight 200–300 g. Both strains were obtained from Møllegaards SPF Avlslaboratorium, Copenhagen. The animals were fed standard rat pellets (Vestlandske Fetteksport, Bergen), giving a sodium intake of approximately 2 mmol/day. Usually food—but not water—was withdrawn in the afternoon the day before the expts. The Sprague Dawley rats and one group of Wistar rats were studied in Inactin (Promonta, Hamburg) anesthesia, given as a single intraperitoneal injection of 120 mg/kg BW. A second group of Wistar rats was anesthetized with Mebumal (pentobarbital), using an initial i.p. injection of 40 mg/kg, and additional i.v. doses of 3–5 mg/kg if needed. The rats were tracheotomized and placed on a heated operating table, the rectal temperature being controlled at 37.0°–37.5°C. For blood sampling and for recording of arterial blood pressure (P_a) a polyethylene tube (PE50) was placed in the iliac artery or the lower abdominal aorta via a femoral artery. A femoral vein catheter was inserted for i.v. injections.

No fluid was given during the expts. except for occasional flushing of the catheters with isotonic saline, usually well below 2 ml in each expt. Varying amounts of saline used for flushing the kidney may have been absorbed from the peritoneum. However, the urine appeared concentrated, and urine flow was low (< 5 µl/min), however measured.

Preparation of the kidney

The left kidney was exposed through a subcostal incision and the ureter, renal artery and vein were dissected free. While some renal nerve strands were severed during dissection, no attempt was made at complete denervation. The kidney was placed in a lucite cup with its dorsal aspect facing upward, and immobilized by cotton moistened in saline. The ureter was cannulated in some expts. When the ureter was left intact, the maintenance of peristaltic waves was checked.

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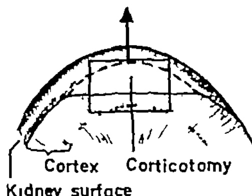
AUKLAND, K., K. HEYERAAS TØNDER and G. NÆSS. *Capillary pressure in deep and superficial glomeruli of the rat kidney.* Acta physiol. scand. 1977 *101* 418–427

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Key words: Bowman's capsule, glomeruli, capillary pressure, glomerular filtration, kidney circulation, renal hemodynamics

While previous indirect estimates of glomerular capillary pressure (P_G) ranged from 60 to 90 mmHg, direct micropuncture of superficial glomeruli in a mutant Wistar rat (Munich strain) has given values of about 45 mmHg (Brenner, Troy and Daugharty 1971, Blantz *et al.* 1972). As a consequence of the latter estimate, the net filtration pressure is low, filtration equilibrium is reached before the end of the average glomerular capillary and the glomerular filtration rate will be strongly dependent on glomerular blood flow (Deen, Robertson and Brenner 1972, 1974). However, recent P_G measurements of 62.6 mmHg in superficial glomeruli which may be occasionally observed on the ventral aspect of the kidney in Sprague Dawley rats (Källskog *et al.* 1975 a) have seriously questioned the validity of the Munich Wistar strain as a representative model. In any case, it is not known whether accessible superficial glomeruli are representative for the whole or a great part of the





2. Kidney injected with silicone rubber ... after corticotomy. Transverse section ... corticotomy made after dehydration ... clearing with methyl benzoate. Viewpoint ... 30° above the long axis of the kidney ... under corticotomy hazy because ... [illegible]

Results

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...he corticotomy might expose as many as 40 glomeruli, and injection of dye and silicone ...ber indicated that blood flow was maintained in practically all visible glomeruli (Fig. 2). ...s was also the case with glomeruli located so close to the incision that they could be ...n to protrude beyond the cut surface. Since these glomeruli were loose and therefore ...ficult to puncture, most measurements were made on glomeruli at an approximate depth ...30 to 100 μm under the corticotomy. The depth of the punctured glomeruli relative ...the kidney surface was approximated by placing a fine forceps with 0.3 mm broad teeth ...tically in the corticotomy and estimating the position of the ablated cortical surface ...der low magnification. The most superficial glomeruli were located 0.15 to 0.20 mm ...der the capsule, while the deepest punctured glomeruli had a depth of 1.3–1.5 mm. ...he silicone rubber preparations indicated that some juxtamedullary glomeruli—giving rise ...o arteriolae rectae—were exposed and might have been punctured. However, since the ...orphology of the punctured glomeruli was not studied, the present measurements do not ...rmit any conclusion concerning pressure in juxtamedullary glomeruli versus deep glomeruli ...of the cortical type.

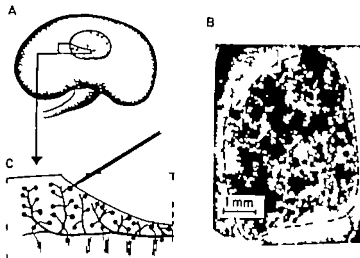


Fig. 1. Corticotomy as viewed from above, A Schematically and B Photographed in vivo. Glomeruli more prominent than in hydropenic kidneys after i.v. infusion of 7 ml isotonic saline. C. Saggital section through corticotomy as indicated in A. Schematic picture of arcuate and interlobular arteries, afferent arterioles and glomeruli.

Pressures were recorded continuously with Hewlett Packard pressure transducers (Model 1280 C) connected to a Hewlett Packard recorder (Model 7811 A). The micropipette measurements were obtained by means of servo-controlled counterpressure ad modum Wiederhielm *et al.* (1964), with a setup slightly modified from that described by Intaglietta, Pawula and Tompkins (1970). (See Acknowledgement at the end of the paper.) The transducers were calibrated before each expt., and zero pressure was checked repeatedly by placing the micropipette in a saline-filled cup at kidney level, or in the "tubular fluid" accumulated in the corticotomy. Since the location of the micropipette within a glomerular capillary cannot be ascertained visually, one has to rely on indirect criteria for acceptance of pressure measurements.

1. The recorded pressure should not change when the feedback gain of the servocontrolled counterpressure system is varied. Because the feedback signal consists of the electrical resistance within and at the opening of the pipette tip, artifactually high pressures are obtained if the pipette tip butts against the vessel wall, and the overestimate will increase with increasing feedback gain.

2. The systolic pressure peak should coincide with or follow within a few milliseconds that of the arterial pressure curve.

3. Pulse-to-pulse and second-to-second variations of arterial pressure should be paralleled in the glomerular capillary pressure.

4. The glomerular pulse pressure curve should reflect characteristics of the arterial pressure, e.g. the dicrotic notch.

5. Injection of dye should not appear in Bowman's space, tubules or interstitium.

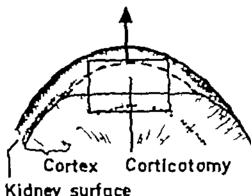
While the first two criteria and in general also no. 3 were satisfied in the reported recordings, criterion no. 4 was not always present and no. 5 was not tested systematically. However pressure recordings deemed absolutely ideal according to the mentioned criteria, did not differ from the rest of the accepted measurements.

The position of the pipette in Bowman's capsule was verified by the appearance of injected dye in the proximal tubuli.

At the end of 3 expts. silicone rubber (Microfil MV 112, Canton Bio-Medical Products, Boulder, Colorado) was injected into the abdominal aorta. When the superficial renal vessels seemed well filled, the kidney was clamped and excised. After dehydration in ethanol of increasing concentrations the organ was cleared in methyl benzoate.

Plasma colloid osmotic pressure was measured directly in arterial blood samples taken at the time of micropuncture by means of the membrane osmometer described by Aukland and Johnsen (1974). Unfortunately many samples were lost during storage and reliable measurements were obtained in only 10 animals.





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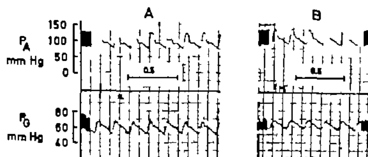


Fig. 3 Original recording of arterial (P_A) and glomerular capillary (P_G) pressures. Glomeruli located 0.6 mm (A) and 0.1 mm (B) under kidney surface.

No measurements were made until at least 15 min after the corticotomy. While the cortical surface often appeared increasingly swollen throughout the expts., glomeruli remained clearly visible and might be punctured for at least 3 h after corticotomy.

Approximate estimates of the rate of fluid accumulation in the corticotomy in a few rats gave values of 1–5 μl/min. Apparent variations in flow rate, and the finding in one rat that dye injected into the renal pelvis appeared at a discrete spot deep in the corticotomy, suggested that reflux of pelvic urine might occur at slight elevations of pelvic pressure. However, the suspicion was not confirmed by measurements of inulin concentrations. Corticotomy fluid/plasma (CF/P) inulin ratios determined in 4 rats varied from 1.4 to 9, with corresponding urine/plasma (U/P) ratios of 188 to 800. Elevation of pelvic pressure to 20 mmHg in one experiment increased CF/P from 1.4 to 5.8, while U/P increased from 2... to 450. In a second rat (saline diuresis), pressure elevation increased CF/P from 3.3 to 3... and U/P from 60 to 82. These observations would seem to exclude appreciable reflux of pelvic urine, and rather indicate that the corticotomy fluid is mainly derived from several proximal tubules, with some slight admixture from more distal nephron segments. This conclusion also agrees with the finding that dye injected into Bowman's space usually emerged in the corticotomy after traversing one or several proximal tubular loops.

Pressure measurements

Satisfactory measurements were obtained in 1 to 7 glomeruli in each rat. Examples of original P_G recordings on midcortical glomeruli are shown in Fig. 3 and 4. As evident from Fig. 4, shortlasting spontaneous variations in P_A were faithfully reflected in P_G, but with a tendency to relatively greater excursions in P_G. A more direct evidence of reactive changes in renal hemodynamics was observed after bolus injection of saline (Fig. 4). Th...

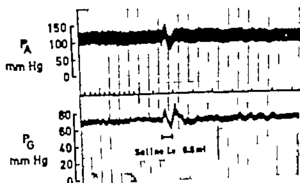


Fig. 4 Original recording of arterial (P_A) and glomerular capillary (P_G) pressures. Glomerulus located 0.4 mm under kidney surface. Time mark ... 10 s.

Table I. Pressures in aorta (P_A), glomerular capillaries (P_G) and Bowman capsule (P_B). Mean ± S.E.

	N*	P_A	P_G	P_B
Wistar, Inactin	18	104.2±2.3 (41)	57.8±1.4 (41)	12.6±1.4 (9)
Wistar, Mebumal	4	108.9±2.7 (13)	58.1±1.3 (13)	10.5±2.0 (10)
Sprague Dawley Inactin	8	109.9±3.0 (14)	58.1±1.7 (14)	10.7±1.1 (11)

N: Number of rats. Number of measurements in parentheses. Pressures in mm Hg.

secondary fall in P_A and P_G was followed by an inproportionate rise in P_G, suggesting dilation of the afferent arteriole as response to the transient pressure reduction.

Provided satisfactory pipette position was maintained, the recorded P_G would usually remain unchanged from the moment of puncture and for a period of up to 30 min. However in some cases the pressure recorded immediately after puncture might be low e.g. 40–45 mmHg, and would then rise to a steady level of 55 to 65 mmHg in the course of 1 to 5 min. In such cases the steady pressure has been presented in figures and tables.

As shown in Table I, average P_G was practically identical in Wistar rats anesthetized with Inactin or with Mebumal, 57.8 and 58.1 mmHg respectively and a similar average (58.1 mmHg) was observed in Sprague Dawley rats anesthetized with Inactin. No difference was observed between punctures made early (15–60 min) or 1 3 h after corticotomy

As evident from Fig. 5 P_G showed no correlation to spontaneous intra and inter individual variations in the arterial pressure level. Furthermore, as shown in Fig. 6, there was no marked difference in P_G between deep and superficial glomeruli. An arbitrary division of glomeruli in three groups according to depth under the kidney surface confirmed this general impression (Table II). However in the Wistar rats the average P_G in the deepest glomeruli (53.8 mmHg) was significantly lower ($p < 0.02$) than that of midcortical glomeruli (60.5 mmHg). The pressures in Bowman's capsule ranged from 6 to 20 mmHg, with an average for all groups of rats of 11.2±0.6 (S.E.) mmHg (Table I)

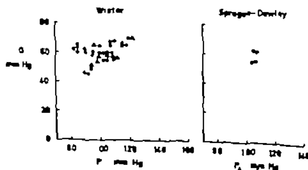


Fig. 5. Glomerular capillary pressure (P_G) in 22 Wistar and 8 Sprague Dawley rats related to corresponding mean arterial pressure (P_A). Open and filled circles: Inactin anesthesia. Triangles: Mebumal anesthesia.

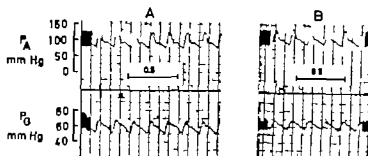


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nd we therefore tend to reject technical-methodological bias as explanation for the high pressures observed in the present rats compared to those reported in the "Munich strain".

In discussing the physiological meaning of the observed glomerular pressures one should keep in mind that all measurements were made 15 to 180 min after surgical intervention on the kidney which might interfere with local hemodynamics in various ways: The apparent swelling of the cut surface might reflect an increased local interstitial pressure which might dilate the afferent arterioles through a myogenic response to reduced transmural pressure (for references see Aukland 1976). The finding of "normal" pressures in Bowman space speaks against a large increase in tissue pressure, but obviously a more complete characterization of the preparation demands studies on autoregulation of glomerular capillary pressure, blood flow and filtration rate.

The corticotomy might well lead to local release of vasoactive substances such as histamine, serotonin, plasmakinins, prostaglandins or renin/angiotensin, but we have no evidence to decide to what extent and in what direction such factors may have influenced the measured P_G. Since inflammatory processes usually have a marked phasic pattern, the observation that P_G did not change systematically with time after corticotomy would to some extent speak against strong influence of local vasoactive substances.

Possible reflux of urine from the renal pelvis into the corticotomy might flood the cortical structures with hyperosmolar solution and give vasodilation. While such reflux was not completely excluded, the finding of corticotomy fluid/plasma inulin ratios of less than 10 even at increased ureteral pressure seemed to exclude appreciable hyperosmolarity.

The observation that most proximal tubules corresponding to the exposed glomeruli were severed by the corticotomy implies that a possible tubuloglomerular feedback mechanism ("macula densa feedback") is disrupted. However, judged from studies on superficial nephrons little change in P_G should be expected. The reflex is mainly activated by increasing distal tubular flow rate,—leading to a fall in P_G— whereas reduction of flow rate from normal level has little or no effect (Schnermann, Persson and Ågerup 1973, Hierholzer et al. 1974).

Probably of greater hemodynamic significance, many interlobular arteries have been cut and sealed by thrombi. Since in most experiments a lense-shaped corticotomy was made, many of the punctured superficial glomeruli might derive their afferent arteriole from intact interlobular arteries, whereas deeper glomeruli most likely would be fed from partly amputated interlobular arteries (Fig. 1 and 2). To the extent that there normally is appreciable resistance, and pressure fall, from the arcuate arteries to the origin of deep and midcortical afferent arterioles, the amputation and resulting flow reduction in the interlobular arteries should lead to an increased pressure in the remaining portion of these vessels. Such "reversed steal effect" of amputation might well be compensated by autoregulatory increased resistance in the afferent arterioles, but if that is not the case, the tendency to lower pressure in deep than in superficial glomeruli (Fig. 6, Table II) might be more pronounced in the intact kidney.

The strongest evidence that "normal" glomerular pressures are obtained with the present technique is the good agreement with the pressures obtained in Sprague Dawley rats in occasional superficial glomeruli punctured through the intact kidney capsule, averaging

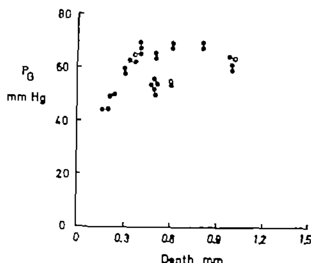


Fig. 6. Capillary pressures (P_G) in glomeru various depth under kidney surface in Wistar (closed circles) and 8 Sprague Daw rats (open circles).

Plasma colloid osmotic pressure at the time of micropuncture averaged 14.3 mm (n = 10). This is considerably lower than the 16–20 mmHg previously observed in Wis rats shortly after Mebumal anesthesia (Johnsen 1974 and unpublished results).

Discussion

The observed glomerular capillary pressures agree well with those recorded by Källsk *et al.* (1975 a) on occasionally occurring superficial glomeruli in Sprague Dawley rats, b are 10–20 mmHg higher than those observed in several studies on the mutant Muni strain of Wistar rats (Brenner *et al.* 1971, Blantz *et al.* 1972). While it is tempting ascribe the discrepancy solely to differences between rat strains, possible methodologic differences should be considered. We believe that the criteria for acceptable measuremen set out above are sound, and technically our recordings would seem to be well comparab to the few previously published records of glomerular pressures. We have been aware th inadequate positioning of the micropipette within the capillary lumen may result in to high values, and high pressure records have therefore been considered with special suspicio However, many pressures of 60–70 mmHg were obtained in technically "ideal" recording

TABLE II. Glomerular capillary pressure (mmHg) at varying cortical depths. Number of glomeruli i parentheses.

	Depth under cortical surface		
	≤0.4 mm	0.5 to 0.9 mm	1.0 mm
Wistar	57.9 ± 1.8 (10)	60.5 ± 1.5 (20)	53.8 ± 2.5* (13)
Sprague Dawley	62.8 ± 2.4 (5)	54.9 ± 2.1 (7)	58.0 (2)

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62.6 mmHg (Källskog *et al* 1975 a) Indirect estimates from proximal tubular stop fl pressure have given varying results, but several investigators have arrived at even high P_G than that observed here (Källskog *et al* 1975 a, Brenner *et al* 1971 and references in the articles)

A P_G of about 45 mmHg, as observed in Munich Wistar rats, indicates that filtrati equilibrium is reached at or just before the end of the glomerular capillaries, *i.e* the plasm oncotic pressure, increased by ultrafiltration balances the transcapillary hydrostat pressure difference (Brenner *et al* 1971 Blantz *et al* 1972) As a consequence, glomerul filtration rate will be strongly influenced by glomerular blood flow (Deen *et al* 1972, 1974 In the present study pressures of 45 mmHg or lower were observed in only 5 out of glomeruli While the filtration fraction was not measured, it seems likely that a net filtra tion pressure of 10 to 15 mmHg would remain at the efferent end of the capillaries most glomeruli, and blood flow variations should only modestly influence GFR (Källsk *et al* 1975 b)

The main new information obtained in the present study would seem to be the similarity P_G in deep and superficial glomeruli While in Wistar rats the deepest glomeruli had low pressure than midcortical glomeruli (Table II Fig. 6), the difference was small, and w would hesitate to attach much significance to these numbers Obviously it would be interest to record P_G in deep glomeruli identified as being of the juxtamedullary type.

The apparently well preserved blood flow and pressure in the exposed glomeruli sugge that the corticotomy technique could be used for micropuncture studies on other dee cortical structures. While not explored so far interlobular vessels might be identified fo pressure measurements, and studies on tubular functions might well be feasible. Th technique is obviously traumatic, but is probably closer to physiological conditions than i vitro studies on homogenates, slices, isolated cells or tubules.

We are greatly indebted to Hans R. Ulfendahl and Mats Wolgast, Biomedicum, Uppsala, S eden, fo patient instruction i micropuncture technique and generous lending of equipment.

We also wish to thank laboratory engineer Erik O Halleland, Institute of Physiology Bergen, Norwa for constructing the mechanical parts of the servo pressure pparatus, and electroengineer Bjarne Hovdene Laboratory for Electronics, Preclinical Institutes, Bergen who modified and constructed the electron feedback system. The modification of the Intaglietta model mainly included more extensive use of integrate components. Detailed description may be provided on request to the uthors.

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Acta physiol. scand 1977 101 428–437

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Catecholamines and Pancreatic Hormones during Autonomic Blockade in Exercising Man

By

H GALBO N J CHRISTENSEN and J J HOLST

Received 13 May 1977

Abstract

GALBO H N J CHRISTENSEN and J J HOLST *Catecholamines and pancreatic hormo[illegible] during autonomic blockade in exercising man* Acta physiol scand 1977 *101* 428–4[illegible]

The import nce of autonomic nervous activity fo the pancreatic hormonal response t exercise I man stud ed 7 men ran at 58 % of V_{O_2} max (determined w thout adm nistratio of drugs) t exhaustion dur α-adrenergic blockade with phentolamine (P), d ring parasympathetic blockade with atropine (A), or [illegible] out drugs (C). At rest phentol m ne creased the plasma concentrations of both insul and norep[illegible] phrine During exercise norepinephrine concentrations increased nd were n P experiments 3 t mes the c[illegible] centrat ons in C experiments. Insulin always declined dur g exercise but in P experiments never decrea[illegible] below basal levels. At identical times neither glucagon nor glucose d ffered significantly in the diffe[illegible] expts. Thus during exercise α-adrenergic blockade increased insuli concentrations but did not d minish [illegible] glucagon response. Nor was th s respo se creased when β-recept r stimulation I P experiments intensif ed by the particularly h gh catecholam ne concentrations. The concentrations of FFA, glycerol a[illegible] lactate were h ghest in P experiments and dentical A nd C experiments. These f ndings ndicate t[illegible] during prolonged moderate exercise n man insulin secretion is depressed by stim lation of α adrener[illegible] receptors whereas glucagon secret on is not influenced by adrenergic receptors. Stim lation of β-adrener[illegible] receptors enhances lipolysis but neither l polysis nor pancreatic hormonal secretion is influenced by chol[illegible] ergic activity during exercise

Key words. Exertion, epineph ne, norepineph ne glucagon, insulin, ympatholytics, tropine phen[illegible] amine, a tonomic nervous system, fatty acids

Current evidence suggests that in several species stimulation of the vagus nerve increases bo[illegible] insulin and glucagon secretion whereas stimulation of the sympathetic pancreatic ner[illegible] diminishes insulin release and enhances glucagon release. A basal tonic discharge from t[illegible] sympathetic as well as the parasympathetic nervous system has been proposed to influence t[illegible] pancreatic A and B cells in the postabsorptive state, and an increased discharge possib[illegible] modulates the function of these islet cells during hypoglycemia. However the role of neu[illegible] mechanisms in the physiologic regulation of insulin and glucagon secretion is unsolved (f[illegible] recent reviews see Day 1975 Gerich, Charles and Grodsky 1976, Unger and Orci 197[illegible] Woods and Porte 1974)

During exercise the activity of the autonomic nervous system changes. Vagal tone, at least on the heart, is reduced (Ekblom *et al.* 1972), and the net activity in the sympathetic nervous system as reflected in plasma catecholamine levels is increased (Galbo, Holst and Christensen 1975). The decrease in plasma glucose concentrations which often occurs during prolonged [illegible] exercise (Galbo *et al.* 1976, Galbo, Christensen and Holst 1977 Harvey Faloona and Unger 1974) may contribute t an activation of autonomic centers in the brain. In the rat adrenergic blocking agents were able to influence the hormonal response to exercise (Harvey Faloona and Unger 1974 Luyckx and Lefebvre 1974). While α-adrenergic blocking drugs diminished the exercise-induced decrease in plasma insulin concentrations (Harvey Faloona and Unger 1974 Luyckx and Lefebvre 1974), the exercise induced increase in glucagon concentrations has been abolished by α- (Harvey *et al.* 1974 Luyckx and Lefebvre 1974) as well as by β- (Luyckx and Lefebvre 1974) adrenergic blocking drugs. I man the pancreatic hormonal response to prolonged exercise, *i.e.* a decrease in plasma insulin concentrations and an initial decrease followed by an increase in glucagon concentrations, was unaffected by β-adrenergic blockade (Galbo *et al.* 1976, 1977). To further elucidate the importance of autonomic nervous activity for the pancreatic hormonal response to exercise in man, we have now measured plasma concentrations of glucagon, insulin, catecholamines and substrates during prolonged exercise which was performed after administration of either the α-adrenergic blocking drug phentolamine, or the anticholinergic drug atropine, or no drugs. The stimulation of cholinergic receptors may inhibit (Colville *et al.* 1964 Weiss and Maickel 1965) and the stimulation of adrenergic receptors promote (Day 1975) lipolysis in [illegible] tissue. The administration in the present study of autonomic blocking agents allowed us to examine also the influence of the autonomic nervous system on lipolysis during exercise.

Methods

[illegible] healthy male students 24 (20–29) yr (mean and range) volunteered in the study. The same subjects had [illegible] the precedent 5 months participated in similar studies in our laboratory (Galbo *et al.* 1976) and [illegible] [illegible] familiar [illegible] the applied procedures. Their mean maximal O_2 uptake ($\dot{V}O_2$ max) determined [illegible] treadmill running (without preceding administration of drugs) was 4 27 (3 39–5 17) l min^{-1} and 5[illegible] ([illegible]) ml kg^{-1} min^{-1}. Mean weight was 77 (63–90) kg and mean height 184 (167 194) cm. Each subject [illegible] in three exercise programs in random order and at intervals of 3 (2 5) week. The subjects [illegible] [illegible] diet throughout the study and avoided physical training for three days before each exercise program. The subjects arrived in the laboratory at 8 o'clock in the morning after good night sleep and at least 10 h [illegible] and tobacco abstinence, in addition to at least 37 h alcohol [illegible]. They were weighed and had [illegible] inserted [illegible] in the forearm, [illegible] they rested in chair for 45 min. The heart rate [illegible] with surface electrodes, both as an electrocardiogram and as cardiotachogram. The cardio-[illegible] and the ECG were continuously displayed on an oscilloscope and periodically recorded through-[illegible] each [illegible]. While the subjects were standing, their expired air [illegible] collected in Douglas bags and blood samples were drawn without stasis. Both gas and blood samples were designated as ($rest_1$). The [illegible] [illegible] air was analysed [illegible] an infrared CO_2-analyser (Beckman LB-1, OA 184) and [illegible] paramagnetic O_2 analyser (Servomex). The accuracy of the analyses [illegible] verified [illegible] the Scholander microtechnique.

On [illegible] the subjects then had 0 035 mg kg^{-1} b wt of atropine (A) injected [illegible] 5 min. 10 min after the start of the injection expired air was collected and blood samples were drawn ($rest_2$-samples). During the subsequent exercise additional 0 015 mg kg^{-1} b wt of atropine were injected [illegible] every 70 min. During exercise the subjects rinsed out their mouth repeatedly [illegible] water, while overheating was avoided by aid of [illegible] and wet towels. 30 min after exercise the persistence of cholinergic blockade was tested by clinical [illegible] and further verified by the lack of effect of 15 mg of metacholine administered [illegible] c.

Another morning the subjects received the dose of phentolamine usually applied in order to establish α-adrenergic blockade (Reinheimer Davidson and Albrink 1968). Immediately after rest -samples had been drawn, 5 mg of phentolamine (P) was administered i v followed by an i.v. infusion of 0.5 mg min⁻ of phentolamine dissolved in isotonic sodium chloride (0 67 ml min). After 40 min of infusion expired air was collected, and blood samples were drawn ($rest_2$-samples). The subjects began to run, and the infusion was maintained, until the last blood samples had been drawn.

In a control-expt. (C) the subjects began to run immediately after the first blood samples had been drawn.

Exercise was performed on a motordri en treadmill, the speed and inclination of which for each subject were identical in the different experiments. The work load was designed to approximate 60% of individual maximal oxygen uptake determined in separate expts. without administration of drugs Immediately after the last blood samples had been drawn at rest ($rest_2$) the subjects began to run during repeated 30 min bouts separated by 10 ml rest intervals. This sequence was continued until exhaustion. Expired air was collected through 2 min after 20 min of each exercise period. In each exercise period, venous blood samples were drawn after 15 min for analysis of glucose, insulin and glucagon, and during the last 2 min for analysis of glucagon, insulin, catecholamines, glucose, glycerol, FFA (free fatty acids), lanine lactate, and hematocrit. Finally blood samples and expired air were collected 30 min after exhaustion. Mineral water was offered when desired.

The methods of sampling and analysis of blood have been reported elsewhere (Galbo *et al* 197C). In respect to glucose concentrations arterialized capillary blood obtained from f ngertips as found not to differ significantly from blood in the cannulated forearm vein. Furthermore, the oxygen saturation of the sampled venous blood was higher than 90 % throughout the experiments. These find ngs indicate that the use of arterial instead of venous samples would not have changed the results. Every subject had all analyses *of a hormone carried out i a gle assay run and the radioimmunoassays* were carried out with antibodies from the same batch as in our preced ng exercise studies. Statistical evaluation of the data as made by means of correlation analysis and by means of Wilcoxon s nonparametric ranking test and the *t*-test for paired comparisons (Snedecor and Cochran 1965) Differences were considered to be significant, if p alues of less tha 0.05 were obtained with both these tests. When nothi g else is stated, concentrations obtained at exhaustion (n = 21) have been used for correlation nalysis. The cited correlation coefficients are signifi cant on the f ve per cent confidence level.

Results

None of the basal values (rest) differed significantly in the three experiments. At rest atropine increased heart rate and serum alanine concentrations and decreased plasma glucose concentrations whereas phentolamine increased heart rate as well as the concentrations of norepinephrine, insulin FFA, glycerol and lactate (Fig. 1–5) The increments at rest in norepinephrine and insulin concentrations correlated significantly (r 0 95).

During exercise oxygen uptake (*57 1 ± 1.5 (A) 58 1 ± 1.3 (P) 57.5 ± 1.3 (C) of individual* V_{O_2} max, *mean and S E.) as well as metabolic rate (kcal/min)* (as calculated from oxygen uptake and R value (respiratory exchange ratio)) were identical during all exercise bouts. The total work time, however was significantly longer in control expts. (175 ± 7 min, mean and S.E.) than in atropine expts. (111 ± 12 min) and phentolamine expts. (108 ± 12 min). Apart from a marked dryness of the mouth in all atropine expts. the subjective experience of exercise varied from individual to individual. In atropine expts. palpitations, irritability nausea and dizziness were reported. In phentolamine expts. palpitations, oppression, dyspnoea, increased tendency to sweating, and pain *in the calves were occasionally* reported, whereas dizziness was not experienced during exercise. At exhaustion in nearly all experiments the subjects reported a feeling of generalized fatigue.

At identical *times during* exercise the glucagon concentrations in plasma did not differ significantly in the three experiments (Fig. 1). After an initial decrease glucagon concentrations subsequently increased progressively in each expt *At exhaustion* glucagon concentra

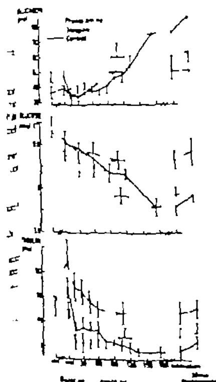


Fig. 1

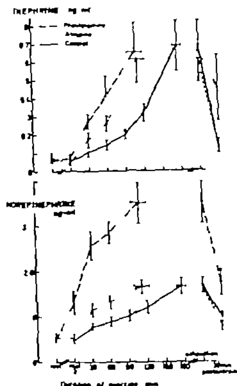


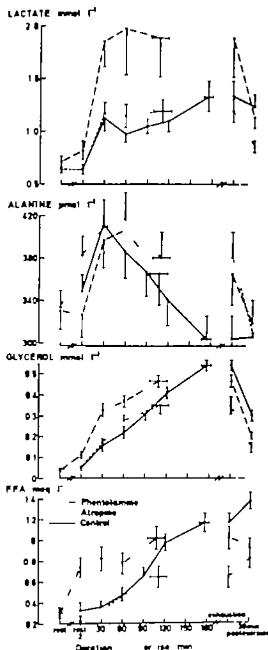
Fig. 2

Mean concentrations (±S.E.) of glucagon, glucose and insulin in plasma obtained during rest and ged exercise are plotted at the work time. When $rest_1$-samples had been drawn, drugs were administ- 7 pmol·l^{-1} of insulin equals one μU · ml^{-1})

Mean concentrations (±S.E.) of plasma catecholamines obtained during rest and prolonged exercise ted at the work time. When $rest_1$-samples had been drawn, drugs ere administered.

were higher in control than in phentolamine expts. (Fig. 1). 30 min postexercise glucagon ntrations did not differ significantly from concentrations at exhaustion and were still e preexercise levels. Glucagon correlated significantly with glucose concentrations 0.6) and ith decreases in glucose concentrations during exercise (r 0.6). Increments acagon concentrations during exercise correlated with glucose concentrations at ex tion (r 0.65) and with decreases in glucose concentrations (r 0.6). Glucagon and ments in glucagon concentrations during exercise correlated with epinephrine (r 0.5 0.6, respectively) but neither with norepinephrine nor with alanine, lactate and FFA ntrations. Insulin concentrations in plasma always declined during exercise (Fig. 1). ng a-adrenergic blockade, however insulin concentrations were significantly higher than tropine and control expts. during as well as after exercise, and never decreased sig- antly below basal ($rest_1$) levels (Fig. 1).

he concentrations of catecholamines in plasma always rose throughout exercise and were identical times lowest in control expts. (Fig. 2). During as well as after exercise the con-



F g 3 Mean concentrations (+ S E) of lacta blood and of lanine, glycerol and free f tty ac serum obtained during rest and prolonged ex re plotted at the work t me When rest -sample been drawn, drugs were adm nistered.

centrations of norepinephrine were highest in phentolamine expts., while epinephrine c centrations at exhaustion were identical in the different expts. (Fig. 2). 30 min postexer the catecholamine levels had decreased but were still higher than preexercise levels highest in phentolamine expts. (Fig. 2) In atropine and control expts. epinephrine correl significantly with the decrease in glucose concentrations during exercise (r 0 65).

The carbohydrate combustion rate (calculated from O uptake and R value measureme was during exercise significantly higher in atropine expts. than in control expts. (represen 55 and 46%, respectively of total caloric output per min (Fig. 4). Plasma glucose c centrations declined gradually during exercise and were during the first 60 min of run identical in the different expts. (Fig. 1). At exhaustion, however the decrease in glucose c

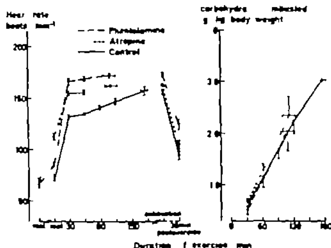


Fig. 4. Mean values (± S.E.) of heart rate and amount of carbohydrate combusted are plotted vs. the work time. When no samples had been drawn, drugs were administered.

centrations below preexercise (rest$_2$) values was significantly largest in control expts., and glucose concentrations were in control as well as in atropine expts. significantly lower than in phentolamine expts. 30 min postexercise glucose concentrations in phentolamine expts. did not differ significantly from preexercise concentrations, while glucose concentrations in control and atropine experiments still were below preexercise levels and still not significantly different from each other.

During exercise blood lactate concentrations were highest during α-adrenergic blockade (Fig. 3). 30 min postexercise lactate concentrations always had decreased below exercise levels, but were still above preexercise values. Alanine concentrations in serum were significantly increased after 30 min of exercise, but no longer at exhaustion, at which time the concentrations were significantly lowest in control expts. FFA and glycerol concentrations in serum gradually increased during exercise and were at identical times highest in phentolamine expts. (Fig. 3). At exhaustion the concentrations in control expts. did not differ significantly from concentrations in phentolamine expts. but were higher than concentrations in atropine expts. 30 min postexercise FFA and glycerol concentrations were still above preexercise levels (Fig. 3).

The exercise heart rate was increased by drugs and to the greatest extent during α-adrenergic blockade (Fig. 4). 30 min postexercise heart rate was still highest in phentolamine expts., in which most subjects were dizzy after exercise. Administration of drugs did not influence hematocrit values at rest. During exercise slight decrease in hematocrit (4 % of preexercise values) took place only in phentolamine and atropine expts.

Discussion

Acute α-adrenergic blockade with phentolamine markedly increased norepinephrine concentrations during exercise (Fig. 2, 5). Similarly schizophrenics treated for months with chlorpromazine, an α-adrenergic blocking agent, had higher plasma norepinephrine con-

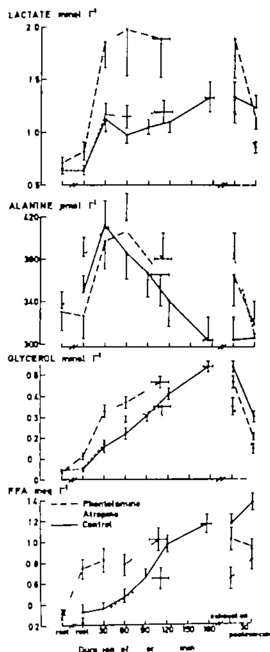


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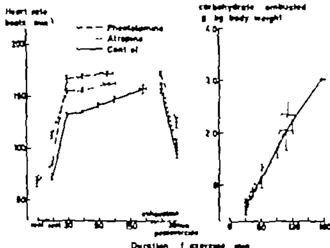


Fig 4 Mean values (±S E) of heart rate and amount of carbohydrate combusted are plotted at the work time. When any samples had been drawn, drugs were administered

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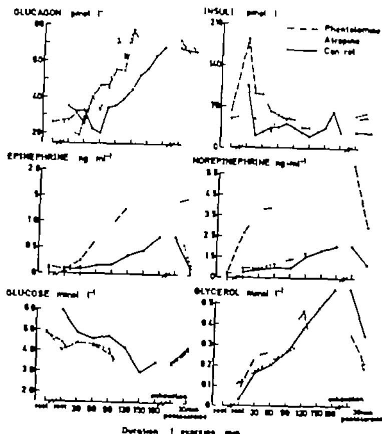


Fig. 5 The entire course of the plasma concentrations of catecholamines, pancreatic hormones, glucose and glycerol obtained during rest and prolonged exercise in one subject (HG). When rest samples had been drawn, drugs were administered.

centrations during exercise than untreated subjects (Carlsson *et al* 1968) These observations may be explained by increased norepinephrine overflow to the blood stream due partly to direct actions of α-adrenergic blocking agents at sympathetic nerve endings leading to an increased norepinephrine concentration within the synaptic gap upon a single nerve impulse (Langer 1974) and partly to increased sympathetic nervous activity Increased sympathetic nervous activity may have been provoked in response to low arterial blood pressures during exercise after administration of α-adrenergic blocking drugs (Carlsson *et al* 1968, Remheimer Davidson and Albrink 1968) Furthermore, since it cannot be excluded that phentolamine reduces V_{O_2} max, the relative load on the working muscles possibly was higher in phentolamine expts than in control expts. even at the same level of oxygen uptake. A higher relative work intensity however cannot completely account for the high norepinephrine concentrations obtained during α-adrenergic blockade, since these concentrations are even higher than the concentrations obtained during treadmill running of maximal intensity (Galbo *et al* 1975). Moreover as indicated by identical carbohydrate combustion rates (Fig. 4) and modest differences with respect to lactate concentrations (Fig. 3) and pulmonary ventilations (53.84 ± 3 95 (P) and 48.55 ± 3.84 (C) 1 (STPD) min mean and S.E., n 7 $p < 0.02$) between phentolamine and control expts. the relative load on the working muscles did not differ much in these expts. (Asmussen 1965)

Also during exercise after administration of atropine, a drug that does not change $\dot{V}_{O_2}$ max (Ekblom et al. 1972), plasma norepinephrine concentrations were higher than in control

expts. (Fig. 2, 5). Acetylcholine released during vagal stimulation has been shown to reduce norepinephrine overflow into the coronary sinus through activation of muscarinic receptors (Langer 1974, Levy and Blattberg 1976). Furthermore, the vagal tone on the heart is partially but not completely withdrawn at a work load of 60% of maximal oxygen uptake (Ekblom et al. 1973). These findings agree with the supposition that abolition by atropine of an acetylcholine induced inhibition of norepinephrine release from cardiac postganglionic sympathetic terminals caused the higher plasma norepinephrine concentrations after atropine in the present expts. The lower epinephrine/norepinephrine ratio obtained at exhaustion in phentolamine expts. compared with atropine and control expts. (Fig. 2) may partly be explained by an increased overflow of norepinephrine from synaptic gaps per nerve impulse during phentolamine infusion (Langer 1974). Since a decrease in plasma glucose concentrations has been shown selectively to enhance the epinephrine response to prolonged exercise (Galbo et al. 1977) also the lower plasma glucose concentrations reached during exercise in atropine and control expts. (Fig. 1) may to some extent account for the higher epinephrine/norepinephrine ratio obtained in these expts. compared with phentolamine expts.

Phentolamine does not influence the secretion of pancreatic hormones from the isolated, perfused pancreas (Iversen 1973). Nevertheless in the present study plasma insulin concentrations were increased at rest by phentolamine (Fig. 1 5). The increments in insulin concentrations correlated significantly with the simultaneous increments in norepinephrine concentrations. These findings suggest that when B cell inhibiting -receptors were blocked by phentolamine at rest, stimulation of pancreatic B cell enhancing β-receptors by increased transmitter concentrations predominated (Gerich, Charles and Grodsky 1976, Harvey et al 1974 Luyckx and Lefebvre 1974). The finding that during exercise insulin concentrations also decreased in phentolamine expts. (Fig. 1 5) in which the increase in catecholamine concentrations was marked, probably was due to the fact that phentolamine is competitive blocking agent and to the existence of more α- than β-adrenergic receptors on pancreatic B cells (Luyckx et al. 1976). However as previously found in rats (Harvey et al. 1974 Luyckx and Lefebvre 1974) throughout exercise plasma insulin concentrations remained higher during adrenergic blockade than in control expts. (Fig. 1 5). In rats the estimated rate of insulin secretion has been found to decrease during exercise, and this decrease could be abolished by adrenergic blockade (Brisson, Malaisse Lagae and Malaisse 1971). Thus a diminished insulin secretion caused by α-adrenergic activity probably accounts for the decreased plasma insulin concentrations found during prolonged moderate exercise in the postabsorptive state (Galbo et al. 1976, 1977 Harvey et al. 1974 Luyckx and Lefebvre 1974), whereas it appears from the present study (Fig. 1) that the parasympathetic nervous system does not significantly influence the insulin response to this kind of exercise. Nor is a decrease in plasma glucose concentrations of major importance for the decrease in plasma insulin concentrations during moderate exercise since we have shown that maintenance of euglycemia by glucose infusion does not diminish the exercise induced decrease in insulin concentrations (Galbo et al. 1977)

Neither α-adrenergic blockade nor parasympathetic blockade abolished the glucagon response to exercise (Fig. 1 5). Nor was the glucagon response enhanced in phentolamine expts. even though the stimulation of β-adrenergic receptors in these expts. probably was

intensified by the particularly high catecholamine concentrations (Fig. 1). It might be argued that differences in plasma substrate concentrations possibly blurred differences in glucagon secretion (Gerich *et al* 1976) However at identical times in the different expts. plasma alanine concentrations were similar (Fig. 3), and we have recently shown that free fatty acids do not significantly influence the glucagon response to exercise (Galbo *et al* 1976). Furthermore in the first 60 min of exercise plasma concentrations of glucose as well as of glucagon were identical whether autonomic blockade was accomplished or not during the last part of exercise the decline in glucose concentrations differed in the different experiments (Fig. 1) but as one would expect (Galbo *et al* 1976, 1977 Gerich *et al* 1976), the rates of decline of glucose concentrations were closely matched to the rates of increase in glucagon concentrations (Fig. 1). Thus in agreement with our previous study of glucagon and plasma catecholamines during β-receptor blockade (Galbo *et al* 1976) the present study could not demonstrate any significant modulating effect of α or β-receptors on the glucagon response to exercise in man. The applied drugs undoubtedly changed the cardiovascular dynamics (Fig. 4) (Ekblom *et al* 1972, Reinheimer Davidson and Albrink 1968). This may have affected the elimination of insulin and glucagon hormones which are both predominantly cleared in the liver and kidney However since the concentrations of insulin and glucagon did not change in parallel during the expts. and furthermore 30 min postexercise still were identical to concentrations at exhaustion, differences in elimination rates of pancreatic hormones caused by effects on the circulation of phentolamine and atropine are not likely to affect the interpretation of the results.

At identical times during exercise plasma concentrations of FFA and glycerol were highest in phentolamine expts. (Fig. 3) This finding indicates according to studies of the relationship between the plasma concentrations of these substances and their rates of production and disappearance at constant work loads (Shaw Issekutz and Issekutz 1975) that lipolysis and muscular uptake of FFA was enhanced during α-adrenergic blockade. Conversely β-adrenergic blockade has been shown to inhibit lipolysis at rest and during exercise (Day 1975 Galbo et al. 1976, 1977). Although muscular uptake of FFA probably was increased in phentolamine expts., as judged from analysis of expired air the rate of fat combustion was not increased However it has been shown that free fatty acids taken up by skeletal muscle are not necessarily oxidized immediately (Zierler 1976) Furthermore in phentolamine expts., as indicated by higher blood lactate levels (Fig. 3) an increased muscular glycogenolysis elicited by increased β-receptor stimulation (Day 1975 Galbo *et al* 1976) possibly exerted a relative inhibition of lipid oxidation The finding that at exhaustion after different work times FFA and glycerol values were identical in spite of higher catecholamine concentrations in phentolamine than in control expts. may be explained by the fact that in control expts. lower plasma concentrations of insulin, which is an antilipolytic hormone, were maintained during a longer work time (Fig. 1 5) The present study was unable to unveil a parasympathetic inhibition of lipolysis (Colville *et al* 1964 Weiss and Maickel 1965). Thus FFA and glycerol concentrations in plasma were not increased during parasympathetic blockade with atropine (Fig. 3 5), a finding which could not be explained by differences in lipid combustion rate (Fig. 4) or in catecholamine and insulin levels between atropine and control expts.

In conclusion the present study indicates that during prolonged exercise in man the secretion

of insulin is depressed by stimulation of α-adrenergic receptors whereas the secretion of glucagon is not significantly influenced by adrenergic receptors. The stimulation of β-adrenergic receptors enhances lipolysis but neither lipolysis nor the secretion of pancreatic hormones are influenced by cholinergic activity during exercise.

Ruth Kall, Rikke Grønbold and Janek Halsted performed skilled technical assistance. Phentolamine was kindly provided by CIBA. Equipment for gas analyses was kindly lent by the Institute of The Theory of Gymnastics, Copenhagen.

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From the Institute of Medical Physiology A, the Panum Institute,
University of Copenhagen, Denmark

K^+-Permeability of the Blood-Brain Barrier, Investigated by Aid of a K^+-Sensitive Microelectrode

By

ANKER JON HANSEN, HENRIK LUND-ANDERSEN and CHRISTIAN CRONE

Received 17 May 1977

Abstract

HANSEN A. J H LUND-ANDERSEN and C. CRONE. *K -permeability of the blood-brain ba investigated bv aid of a K sensitive microelectrode* Acta physiol. scand. 1977 438–445

The K -permeability f the blood-brain barrier at the capillary level was estimated from determi of brain extracell lar K -concentration in response to an isotonic bolus containing KCl injected in carotid artery A very low permeability ppeared from the fact that the extracellular K -concentrat measured by aid of K -sensitive microelectrodes—remained unchanged duri g the passage of the l An upper limit for the blood-brain barrier K -permeability in the rat was estimated to be 2.8 10⁻ c

The extracellular K -concentration in the brain is kept approximately constant de considerable and long lasting changes of the plasma K -concentration (Bradbury and K man 1967 Cohen *et al* 1968). However during spreading depression, anoxia or sei large transient changes of the extracellular K -concentration occur (Vyskočil *et al* 1 Prince *et al* 1973 Hansen 1977) The K permeability of the blood-brain barrier is impo for these phenomena since brain extracellular K -concentration might be influence passage of K between brain and blood across the barrier Also an exact knowledge o K -permeability of the blood-brain barrier would be important for a thorough analys the DC potential between brain extracellular fluid and blood

So far two methodological approaches, the tissue uptake technique (Katzman and Le man 1953 Bradbury and Kleeman 1967) and the indicator diffusion technique (Crone Thompson 1970) have been applied to study the K -permeability of the blood-brain ba These studies have revealed a very low permeability However a detailed quantitative i pretation of the data obtained with these two methods in terms of blood-brain ba permeability at the capillary level is difficult for slowly penetrating solutes since trans occurs across many interphases besides the blood brain barrier· the arachnoid memb the choroid plexus and structures lacking a blood-brain barrier The indicator diffu technique has the particular complication that the test solute (⁴²K) and the reference stance may be separated intravascularly by Taylor s effect (Lassen and Crone 1970).

This complication is particularly disturbing when dealing with substances which permeate only slowly through the blood-brain barrier because of the difficulty of distinguishing between intravascular separation of test solute and reference substance and a small separation due to a minute transcapillary permeation of the test solute.

Davson and Welch (1971) approached the problem of a quantitative assessment of the K-permeability of the blood-brain barrier by fitting a mathematical model to brain and CSF uptake data. However ingenious, this approach remains indirect, and the permeability data are necessarily encumbered with some uncertainty since several parameters of the model simulating the entire system are not yet known with great accuracy.

The introduction of K^+-sensitive microelectrodes (Walker 1971) permits a more direct determination of capillary permeability to K^+ which we have employed in the present study.

A preliminary report was presented at the XVth meeting of the Scandinavian Physiological Society (Hansen and Lund-Andersen 1976).

Methods

Operative procedure. The experiments were performed on 27 adult male Sprague-Dawley rats weighing 250–300 g. The animals were anesthetized with pentobarbital administered i.p. (50 mg/kg) and a cannula was placed in the trachea. One catheter (pp 25) was retrogradely guided to the arch of the aorta through the abdominal aorta. Another catheter (pp 10) was placed in the external carotid artery with the tip situated near the carotid bifurcation (Murray and Ploplys 1972). The head of the rat was mounted in a head holder and the calvarium cleared. The sagittal sinus was exposed through a burr hole in the midline 3–4 mm in front of the coronal suture. The dura and the connective tissue overlying the sinus was carefully dissected so that the blood stream was visible. Through another burr hole the parietal cortex was partially exposed and a small incision was made in the dura without any damage to the pia. Two small silver–silver chloride electrodes were placed diametrically opposite around the burr hole for recording of the EEG.

The animals were paralyzed with gallamonium iodide and artificially ventilated at settings to maintain normal blood gases and pH. The blood pressure was monitored via the catheter in the aorta. The rectal temperature was kept constant at 37°C by a servocontrolled heating system.

K^+-sensitive microelectrodes. The potassium concentration in the extracellular fluid and in the sagittal sinus was measured with K^+-sensitive microelectrodes. The manufacturing and use of double-barrelled glass micropipettes has previously been described (Hansen 1977). The tip diameter was about 1 µm and the impedance of the barrel containing potassium ion exchanger (Corning 477317) was 10^8–10^9 Mohm.

One electrode was placed in the sagittal sinus. Another electrode was positioned in the parietal cortex through the slit in the dura. The tip of the electrode was located 200–700 µm below the surface by means of a step-micromanipulator. In a few cases the electrode was placed in the extracellular fluid of neck muscle or the mesentery. The animals were grounded via glass tubes filled with 150 mM KCl solidified agar and buried in a neck muscle. The experimental set-up is shown schematically in Fig. 1. The voltages from the electrodes were recorded by a high resistance DC-amplifier with input resistance of 10^{15} Mohms. For each electrode the potential difference between the two barrels indicated the K^+-concentration, the relation between K^+-concentration and potential being approximately logarithmic with a slope of 40–50 mV per 10-fold change in K^+-concentration. The voltage between the reference barrel of the cortex electrode and the common ground measured the DC-potential between brain extracellular fluid and ground. All parameters were recorded on a multi-channel UV-recorder (Southern Instruments).

The K^+-electrodes were found to be very sensitive to gallamonium iodide as they are sensitive to tetraethylammonium chloride (Neher and Lux 1973); this complicated the administration of gallamonium. Thus, 15–20 min had to elapse between the initial administration of gallamonium and placement of the electrodes. When additional gallamonium was needed the sinus electrode was removed and replaced after this time period. When this procedure was followed the sinus electrode measured the plasma K^+-concentration satisfactorily cf. the plasma K^+ of about 5 mM (Fig. 2). The electrode in the brain was unaffected by gallamonium administration indicating that this substance does not pass the blood–brain barrier.

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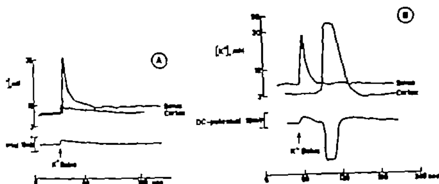
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. The course of the K -concentration in the sagittal sinus, the K -concentration in brain extracellular and the DC-potential between brain extracellular fluid and ground. The figures show some atypical ... upon KCl injections. The left part (A) illustrates situation where minor increase of brain ...cellular K -concentration occurred. The right part (B) illustrates situation where the extracellular ...concentration remained constant whilst the bolus passed the brain, but 35 later the K -concentration and the DC-potential changed as during spreading depression

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...her tissues

...order to examine the response in organs with a significant capillary K -permeability ...pts. were performed on striated muscle and mesentery Fig. 4 A shows the K -concentration in the extracellular fluid of a neck muscle and in the sagittal sinus in response to a bolus ...ection through the aortic catheter Contrary to what was seen in the brain the bolus ...passage was clearly reflected in the extracellular fluid of the muscle. The expts. were some...what disturbed by muscle contraction due to the potassium-depolarization and were only ...successful in 2 out of 12 animals. In the mesentery however it was easy to demonstrate ...a large increase in the extracellular K -concentration when a bolus was injected through a

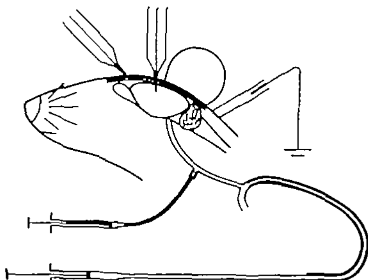


Fig. 1 Schematic presen the experimental set-up. ' ble-barrelled K -sensitiv electrodes are positione sinus and in the extracell of the brain. One catheter in the proximal part of the carotid artery while anot eter is led from the al aorta to the arch of aorta

Injection through the catheters Bolus es of 75–200 μl KCl-solutions in phosphate buffer (pH 7.4 were injected through the catheters over 1 2 s. In the aortic catheter 1 M KCl solutions were u 60–150 mM solutions were used in the carotid catheter NaCl-solutions of similar tonicity served as Results obtained when the KCl-solutions were injected either through the carotid or the aortic were indistinguishable and the d ta will accordingly be described and discussed together

Results

Brain

Fig. 2 shows the K -concentration in the sagittal sinus and in brain extracellular together with the DC-potential between sinus and ground or brain and ground. The illustrates the typical response when a KCl-bolus was injected either through the car the aortic catheter It appears that the K -concentration in the sagittal sinus varied as a typical organ outflow curve after intra-arterial injection, indicating that the bol

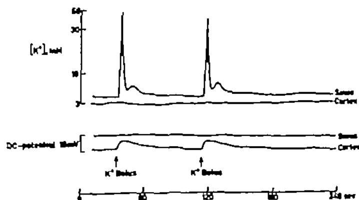


Fig. 2. Time course of the K$^+$-concentration in the sagittal sinus, the K -concentration in brain extrac fluid the DC-potential between reference barrel of the sinus electrode and ground and the DC-potent tween brain extracellular fluid and ground. 75 μl of a 150 mM KCl solution was over 1 2 s peri jected through the carotid catheter A small recirculation peak ppears i the sinus. Note that the cha the brain-ground DC-potential only slowly declined after the bolus had passed the brain. When the potential was normalized after 80 s the injection of KCl was repeated.



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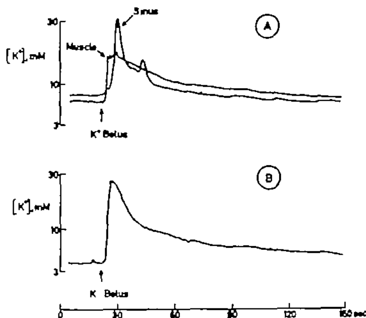


Fig 4 (A) Time course of the K concentration in the sagital sin and in the extracellular fluid of neck muscle when a KCl-bolu was injected through the catheter with the tip placed in the aort arch. (B). Time course of the K concentration in the extracellular fluid of the mesentery when a KC bolus was injected through cath eter with the tip placed near th coeliac trunk

catheter in the abdominal aorta (Fig. 4 B) Intracellular location of the electrodes can b excluded since the DC potential between reference barrel and ground was close to 0 mV These expts. clearly show that in tissues with a relatively high K permeability of the capi laries changes of the plasma concentration are rapidly and significantly transmitted to th extracellular fluid

Discussion

The constancy of the extracellular K -concentration when a KCl containing bolus pass the brain leaves little doubt that the blood brain barrier is close to being impermeable t potassium The sources of error in the experimental design are few The electrode tip view the events in a minute region somewhere between the capillary and the cellular structures o the neuropil. Since the mean intercapillary distance is about 30-40 μm (Diemer 1968) n passage of potassium into a local brain region from a capillary should rapidly be monitore by the electrode, as illustrated in muscle and mesentery Other sources of error include arre of the local capillary perfusion by the electrode. This is, however unlikely since the extra cellular K -concentration around the electrode tip remained low throughout an experimen indicating sufficient supply of blood to the neighbouring cells. Another complication woul be arteriolar smooth muscle contraction due to the depolarizing effect of the high pota sium in the bolus. The rapid passage of the bolus indicates, however that there was no flo interference. This possibly signifies that the permeability of the endothelium in arterioles similarly low

Even though uncontrollable experimental errors seem to be small we cannot conclu that the blood-brain barrier has zero permeability to potassium since there is a lower lim to the electrode response. Thus, instead of postulating zero permeability we have calculate an upper limit for the permeability using the following reasoning.

Derivation of a permeability coefficient of the blood-brain barrier to potassium

ue to spontaneous small potential fluctuations observed with the cortex-electrode changes s than 0.5 mV were regarded as insignificant. A 0.5 mV change corresponds in the 3 mM otassium region to 0.1 mM change in K⁺-concentration. Thus, the apparent constancy of e brain K⁺-concentration during a bolus passage through the vascular system only safely dicates that the K⁺-concentration in the extracellular fluid did increase less than 0.1 mM This means that in a hemisphere weighing 1 g with an extracellular space of 15% less than $15 \cdot 10^{-4}$ mmol K⁺ has passed the blood-brain barrier disregarding uptake of K⁺ in brain cells. In expts. where KCl was injected through the external carotid artery the amount of potassium supplied to the hemisphere on the side of injection can be calculated from the time-concentration curve recorded in the sagittal sinus as F (1 − Hct) . A, where F is blood flow Hct is hematocrit—F (1 − Hct) is thus the plasma flow—and A is the integral of the time-concentration curve (μmol ml⁻¹ s). This last value is multiplied by 2, because the concentration in the blood perfusing the hemisphere on the injected side is approximately twice as high as after the dilution in the sinus with a similar amount of blood from the non-injected side. The area under the potassium curve in the sagittal sinus is 110 μmol ml⁻¹ s in the example shown in Fig. 2. With a Hct of 0.45 and a blood flow of 1/60 ml g⁻¹ s⁻¹—the normal value for rat cortex under well-ventilated conditions—these figures yield a maximal extraction, E, of 0.0015/(1/60·0.55·2·110)·100 = 0.74%. However, since the Hct on the injected side is lowered due to the addition of the red cell free bolus the value of E is further reduced. If the Hct were lowered to 0.40 unilaterally E would be 0.68%. If it were 0.35 E would be 0.63 ... In the subsequent calculations we chose a Hct of 0.40. Our value for K⁺ permeability for this reason alone would be an upper estimate.

There are previous reports of potassium extraction during a single capillary passage in the brain. Crone and Thompson (1970) reported a ⁴²K-extraction of 2.2%, a figure which is clearly too high. The explanation of this falsely high figure is intravascular separation of potassium from Evans Blue Dye which was used as reference solute and transendothelial loss in the choroid plexus and blood-brain barrier free areas. Lassen *et al.* (1971) using ⁴²Na ... then arrived at an extraction of 1.4%, a figure which is most likely too high, again due to the same complications, although they carefully corrected for Taylor-effects. Available correction methods in cases with very low extraction, are however of limited accuracy.

With knowledge of the extraction a potassium permeability can be calculated from P = −(F/S) (1 − Hct) ln (1 − E) where S is capillary surface area (240 cm²) in 1 g of brain (Crone, 1965). Under the proviso that the potassium permeability is independent of concentration P = ... as $2.8 \cdot 10^{-7}$ cm sec⁻¹. This value is two orders of magnitude smaller than that for muscle capillaries (Renkin and Sheehan, 1970), illustrating the extremely low permeability of the blood-brain barrier.

Davson and Welch (1971) approached the quantitative assessment of the potassium permeability by modelling whole brain data from Bradbury and Davson (1965) and from Bradbury and Kleeman (1967) and taking into account the extra supply of ⁴²K from the choroid plexus. Using the same capillary areas as we have used their model gave a P_K of $5.6 \cdot 10^{-}$ ... cm ... for efflux across the blood-brain barrier. In view of the heterogeneous nature of the

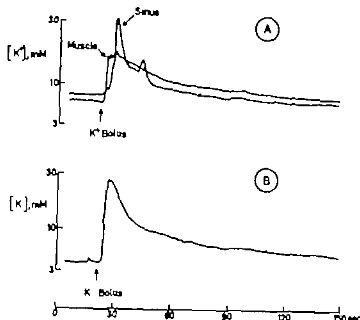


Fig. 4 (A) Time course of th[e] concentration in the sagittal [sinus] and in the extracellular flui[d of] neck muscle when a KCl [bolus] was injected through the ca[theter] with the tip placed in the [aortic] arch. (B) Time course of th[e] concentration in the extrace[llular] fluid of the mesentery when [a] bolus was injected through a [cath]eter with the tip placed nea[r the] coeliac trunk

catheter in the abdominal aorta (Fig 4 B) Intracellular location of the electrodes ca[n be] excluded since the DC-potential between reference barrel and ground was close to 0 [mV]. These expts. clearly show that in tissues with a relatively high K permeability of the ca[pil]laries changes of the plasma concentration are rapidly and significantly transmitted to [the] extracellular fluid.

Discussion

The constancy of the extracellular K -concentration when a KCl containing bolus pa[sses] the brain leaves little doubt that the blood-brain barrier is close to being impermeabl[e to] potassium. The sources of error in the experimental design are few The electrode tip vi[ews] the events in a minute region somewhere between the capillary and the cellular structure[s of] the neuropil Since the mean intercapillary distance is about 30-40 μm (Diemer 1968) [a] passage of potassium into a local brain region from a capillary should rapidly be monito[red] by the electrode, as illustrated in muscle and mesentery Other sources of error include ar[rest] of the local capillary perfusion by the electrode. This is, however unlikely since the ext[ra]cellular K -concentration around the electrode tip remained low throughout an experim[ent] indicating sufficient supply of blood to the neighbouring cells. Another complication wo[uld] be arteriolar smooth muscle contraction due to the depolarizing effect of the high pot[as]sium in the bolus. The rapid passage of the bolus indicates, however that there was no fl[ow] interference. This possibly signifies that the permeability of the endothelium in arterioles [is] similarly low

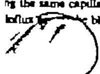
Even though uncontrollable experimental errors seem to be small we cannot conclu[de] that the blood-brain barrier has zero permeability to potassium since there is a lower lim[it] to the electrode response. Thus, instead of postulating zero permeability we have calculat[ed] an upper limit for the permeability using the following reasoning.

taneous small potential fluctuations observed with the cortex-electrode changes mV ere regarded as insignificant. A 0.5 mV change corresponds in the 3 mM gion to 0.1 mM change in K -concentration. Thus, the apparent constancy of -concentration during a bolus passage through the ascular system only safely t the K -concentration in the extracellular fluid did increase less than 0.1 mM that in a hemisphere weighing 1 g with an extracellular space of 15% less than of K has passed the blood-brain barrier disregarding uptake of K in brain ts where KCl was injected through the external carotid artery the amount of pplied to the hemisphere on the side of injection can be calculated from the ration curve recorded in the sagittal sinus as F (1 − Hct) 2A, where F is blood hematocrit—F (1 − Hct) is thus the plasma flow—and A is the integral of the ration curve (μmol ml⁻ s). This last value is multiplied by 2, because the n in the blood perfusing the hemisphere on the injected side is approximately as after the dilution in the sinus with a similar amount of blood from the non- . The area under the potassium curve in the sagittal sinus is 110 μmol ml⁻ s le shown in Fig. 2. With a Hct of 0.45 and a blood flow of 1/60 ml g⁻¹ s⁻ —the for rat cortex under well-ventilated conditions—these figures yield a maximal of 0.015/(1/60 0.55 2 110) 100 = 0.74%. However since the Hct on the in- lowered due to the addition of the red cell free bolus the value of E is further he Hct were lowered to 0.40 unilaterally E would be 0.68 %. If it were 0.35 E 53% In the subsequent calculations we chose a Hct of 0.40. Our value for K for this reason alone would be an upper estimate.

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nd Welch (1971) pproached the quantitative assessment of the potassium per modelling whole brain data from Bradbury and Davson (1965) and from Brad- leeman (1967) and taking into account the extra supply of ⁴²K from the choroid ng the same capillary areas as w have used their model gave a P_K of 5.6 10⁻⁸ influx the blood-brain barrier In view of the heterogeneous nature of the



entire blood-brain-CSF system our results lend a valid support to the Davson and Welch data and a slight revision in the downward direction Our upper limit for the potassium permeability brings this figure closer to the figures for sodium and chloride permeability given by Davson and Welch in the same paper 0.9 10 cm s^{-1}

The figure for P_K of 1 4 10⁻ cm s⁻ reported by Rapoport (1976) results from a recalculation of Davson and Welch s figure for P_K of 5 6 10⁻ cm s Rapoport derived t½ of 68 min from this figure by assuming that the entire brain could be treated as a single compartment with a fractional volume of distribution for K of 0 8. This is hardly correct since the apparent volume of distribution for K is much larger than 0 8 due to the high intracellular K -concentration. Using a half-time of 68 min Rapoport derives a P_K for the passage from plasma to extracellular fluid of brain (fractional volume of 0.2) of 1 4 10 cm s (0.2/0 8 5 6 10 ⁷). This method of recalculating Davson and Welch s data is erroneous for the reason mentioned and Davson & Welch s figure cannot be changed unless the model is reformulated

Our value of P_K for the blood-brain barrier of 2.8 10^{-7} cm s (per cm capillary surface) represents the as yet lowest experimentally determined permeability The 0.5 mV detection limit, the capillary area of 240 cm /g for cortex and the hematocrit of 0 40 are likely to cause overestimation of P_K and it must, therefore, be emphasized that although this P_K is very low the true P_K may well be even lower It is, therefore, not yet possible to know with certainty whether P_K and P_{Cl} are different or similar Such knowledge would have more than academic interest in view of its importance for the understanding of the genesis of the DC-potential across the blood-brain barrier

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Acta physiol. scand 1977 101 446–457

From the Institute of Medical Physiology C, University of Copenhagen, Denmark

Sustained Water Diuresis in Anesthetized Dogs Antidiuresis in Response to Intravenous and Bilateral Intracarotid Infusion of Hyper-Osmolar Solutions of Sodium Chloride

By

PETER BIE

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Abstract

BIE, P *Sustained water diuresis in anesthetized dogs Antidiuresis in response to intra and bilateral intracarotid infusion of hyper-osmolar solutions of sodium chloride* physiol scand 1977 *101* 446–457

The function of the suggested hypothalamic osmoreceptors was re-examined in dogs during light chlo anesthesia. The body weight of the dogs was increased to—and mai tained at—102.0% of the initial by i infusion of a hypo-osmolar solution of glucose and urea Duri g the water diuresis renal free clearance (C_{H_2O}) remained approximately constant for more than 3 h during which infusion of physiol amounts of vasopressin (5 or 10 μU/(kg b.wt. min)) was associated with a dose-dependent, reve decrease in C_{H_2O}. Infusion of hyper-osmolar sodium chloride solution (90 μmol/(kg b.wt min) for 60 was performed either through central venous catheter or through needles placed in both common ca rteries. After an initial increase in C_{H_2O} these infusions elicited antidiuresis (negative C_{H_2O}) and inc in the rates of excretion of sodium (100-fold) and potassium (3-fold). Left atrial pressure nd renal clea of inulin and PAH did not change significantly The renal effects of NaCl infusion were independe the mode of infusion It is concluded that the results are inconsistent with the hypothesis that the supplied by the common carotid arteries ncludes an osmoreceptor which is capable of changing — seconds—the rate of secretion of vasopressin, although they support the view that the renal excretic water is strongly influenced by a receptor which is sensitive to osmolality or concentration of sodium addition it appeared that an increase of few per cent in the concentration of sodium in plasma socialted with substantial increase in the rate of excretion of sodium without measurable change central venous pressure or in glomerular filtration rate.

It is generally accepted that the rate of excretion of water is largely determined by the c centration of vasopressin in blood plasma This concentration however is known to influenced by many factors of which only the osmolality and the volume of some ext cellular compartment are supposed to be elements of the regulation mechanisms wh maintain the composition and volume of the extracellular fluid within physiological lim Changes in blood or extracellular fluid volume can be detected by stretch receptors plac within suitable structures *e.g* the atrial wall. In their critical review Goetz, Bond and Bl ham (1975) concluded, however "that the left atrial volume-receptor hypothesis ad

to our current understanding of the regulation of the extracellular fluid balance" and pointed out some measure of concentration as the variable to which the rate of secretion of vasopressin is adjusted under physiological circumstances. The physiological importance of this regulatory mechanism—called an "osmoreceptor"—seems indisputable [illegible] Shelton and Athar 1976). In contrast to Verney's interpretation of his results [illegible] (1947) recent evidence (Ble 1976 a) indicates that the receptor is not situated within [illegible] supplied by the carotid arteries. In the latter expts. no antidiuresis was elicited by [illegible] into the carotid arteries of hyper-osmolar sodium chloride solutions in dosages [illegible] upon i.v. administration neither restored plasma osmolality to normal nor elicited [illegible]. The present experiments were designed to evaluate the validity of these results answering the following question. Does antidiuresis develop when the concentration of [illegible] in plasma of the anesthetized water-diuretic dog approaches normal values as a [illegible] of i.v. administration of hyper-osmolar saline? and—if so—is the antidiuretic response to i.v. infusion different from the response to bilateral intracarotid administration of hyper-osmolar sodium chloride solution at the same rate?

Methods

[illegible] beagle bitches weighing approximately 10.8 kg (range 9.5–12.2 kg) were used. They were [illegible] commercial dog diet and regular measurements of the daily intakes of sodium and potassium [illegible] of 4.1 [illegible] 0.1 and 3.5 [illegible] 0.1 mmol/kg b.wt., respectively (mean ± S.E. [illegible] = 81). Before the present [illegible] surgical dislocation of the carotid arteries (Muchee 1972) was performed in order to facilitate [illegible] puncture. Each animal was used for [illegible] number of expts. [illegible] intervals of at least 2 weeks.

General procedure. Prior to experiments food was withheld for 18 h. The dog had free access to [illegible] Atropine (40 µg/kg b.wt.) was given [illegible] 1/2 h before induction of anesthesia. A sterile through-[illegible] catheter [illegible] introduced into [illegible] lateral saphenous vein and after collection of [illegible] blood sample [illegible] anesthetized with chloralose (60–70 mg/kg b.wt.). Throughout the expt. supplementary doses [illegible] 20 mg/kg b.wt. [illegible]) were given. The chloralose was purified as described earlier (Ble 1976 [illegible]) [illegible] (10 g/l) in [illegible] solution of glucose (40 mM) and urea (25 mM). This solution of glucose and [illegible] referred to as GU-solution. Small amounts of pentobarbital (5 to 10 mg/kg b.wt. [illegible]) proved [illegible] effective in preventing excitation during induction and was given in all experiments. Following [illegible] another through-the-needle catheter was placed in or near the right atrium after [illegible] of the right external jugular vein. Appropriate priming doses of ^{14}C-inulin and ^{3}H-p-aminohippurate (NEN Chemicals, Dreieichenhain, W. Germany) were then injected and an infusion of these [illegible] was started. [illegible] expts. of series [illegible] (see below) puncture of both carotid arteries was performed [illegible] earlier (Ble 1976 a). After insertion of [illegible] Foley catheter into the urinary bladder the dog was [illegible] to the experimental set-up of which a diagram is shown in Fig. 1. This includes [illegible] control [illegible] load cell—electronics—pump A—animal/load) capable of controlling the body weight of the [illegible] within [illegible] 0.2 [illegible] of the desired value (Ble 1976 [illegible]). Zero adjustment of the control current of the servo [illegible] and recording of the central venous pressure was performed between 10.00 and 10.10 [illegible] At 10.10 collection of urine and the infusion of GU-solution was started (pump B, Fig. 1). 20 ml/kg b.wt. was [illegible] during 60 min. From Fig. 1 can be seen that the function of pump B did not influence the control [illegible] which was activated only by [illegible] decrease in the total load [illegible] by evaporation and diuresis. Including [illegible] period of infusion of GU-solution (0–60 min) the experiments took 4 h, the test solution containing [illegible] or NaCl [illegible] infused during the third hour (120–180 min corresponding to 00.10–01.10 p.m.). [illegible] which could not be performed according to the schedule were discarded. All expts. were [illegible] similar except for the composition, the volume, and the mode of administration of the infusate [illegible] by pump D (Fig. 1). Five series of experiments were performed.

[illegible] Control expts. In these no test solution was infused and clearances of inulin and PAH were [illegible] measured [illegible] pumps C and D (Fig. 1) [illegible] not in use.

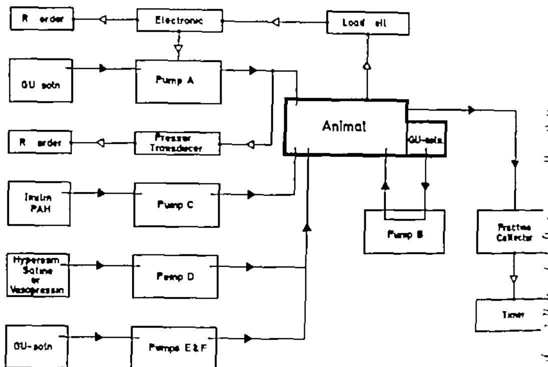


Fig. 1 Diagram of the experimental set-up. *Filled arrows* indicate routes of fluid flow *Open arrows* indica directions of actions which were electrical except f the gravitational input to the load cell and the inp to the pressure transducer The weight of the dog and a reservoir contain g GU-solution (I the weig of components of the double-contoured bo) was registered by the load cell and sustained by the action load cell, "electronics and pump A. For the sake of simplicity the function of the timer and the temper ture controlling systems have been excluded. The timer stopped the pump(s) infusing through the cent venous catheter and simultaneously activated the recorder f r a period of 40 s every time the fracti collector made step *Le* every 10 min. Pumps E and F were used in series only

Series b I infusion (0.2 ml/min for 60 min) of vasopressin (5 μU/(kg b.wt. min)) dissolved in GU solution. Clearances of inulin and PAH were not measured.

Series c. I infusion (0.2 ml/min for 60 min) of vasopressin (10 μU/(kg b.wt. min)) dissolved in GU solution.

Series d. I v infusion (0 4 ml/min for 60 min) of hyper-osmola sodium chloride solution (90 μmo (kg b.wt. min)). The concentration of N Cl was approximately 2.2 M

Series Bilateral intracarotid infusion of hyper-osmolar sodium chloride solution similar to that giv in series *d*, 0.2 ml/min was given into each artery Before and after this infusion the cannulae were kept op by infusion of GU-solution (approximately 0.1 ml/min per artery pumps E and F in Fig. 1).

Central venous pressure was measured every 10 min by a strain gauge transducer (Model P23BB, St ham Laboratories, Hato Rey Puerto Rico) and an UV recorder (Model 3006, S.E. Laboratories, Feltha England). The oscillations reflecting the heart beats were eliminated by an electronic filter which allow the deviations caused by the respiratory activity to be seen The maximal value during expiration w taken as the central venous pressure.

During anesthesia blood samples (3 ml) were obtained through the central venous catheter at times i dicated in Fig. 2, sampling was each time followed by njection of an equivalent volume of dextran (Macr dex®-saline, Pharmacia, Uppsala). Coagulation was prevented by dry heparin and centrifugation k separation of plasma was started 1 2 min after sampling.

The initial injection of chloralose and all infusions through the central venous catheter were perform through sterile filters (Millex® 0.22 μm, Millipore Corp Bedford, Mass.). The rectal temperature of t conscious animal was measured in the morning and throughout the expt. this temperature was kept clo to its morning value (±0.2°C) by heating of both the operating table and the fluids delivered by pum A and B.

Vasopressin. The USP Posterior Pituitary Reference Standard was prepared and stored as recommended by [illegible] (1969). The extract was diluted with GU-solution immediately before use. The rates of infusion was equivalent to 13 · $10^{-[illegible]}$ and 25 · $10^{-[illegible]}$ mol arginine-vasopressin/(kg b.wt. · min).

Analytical methods. Haematocrit was determined in quadruplicate immediately after sampling by centrifugation for 5 min at 20 000 *g* using micro-haematocrit capillaries. Osmolality was determined by an osmometer (Advanced Model 31 LAS) as described earlier (Bie 1976 a). On the day of the expt. the concentrations of Na^+ and K^+ in samples of plasma and urine were measured by means of a flame photometer (IL 3[illegible], Instrumentation Laboratory Inc., Lexington, Mass.) using lithium as internal standard. When plasma was analyzed the apparatus was calibrated by use of the viscosity-adjusted standard solution provided by the manufacturer. Dilution by a roller pump has been described as a potential source of methodological error (Vader and Vink 1975). The rates of suction of standard solution, dog plasma, and water were measured by accurate densitometry (Kratky, Leopold and Stabinger 1969) and repeated weighing. The values for plasma and standard solution showed no significant difference (both were about 1% lower than the value obtained for water). Thus it was assumed that the results were uninfluenced by the use of the roller pump belonging to the flame photometer.

Samples of plasma and urine were deep-frozen until the concentrations of ^{14}C and ^{3}H were determined in a liquid scintillation counter (Liquid Scintillation System, Mark II, Nuclear-Chicago) using PCS® (Nuclear-Chicago) as scintillation fluid. The amounts of ^{14}C-inulin and ^{3}H PAH infused into the animals was adjusted so as to produce a ratio between ^{3}H and ^{14}C of about 10:1. In separate expts. the concentration of water in plasma during water diuresis was found to be 93.1% (v/v). Therefore, the clearance of inulin was multiplied by 0.931 in order to obtain glomerular filtration rate. The rate at which Na was filtered into the renal tubules was calculated as *glomerular filtration rate times the concentration of Na in plasma.* The concentration of glucose in plasma was determined in several expts. by the method described by Werner, Rey and Wielinger (1970), the values after hydration with GU-solution (mean 7.03 mM, S.E. [illegible] mM, *n* = 30) were not different from those measured in plasma obtained before hydration (mean 7.43 mM, S.E. 0.34 mM, *n* = 20). The urine was examined by use of N-Labstix (Ames Co., Stoke Poges, England) before and during the experiments. Protein, glucose, ketone bodies, and bacteria were never detected in the urine but traces of haemoglobin were occasionally present.

Recovery. The recovery after chloralose anaesthesia was rapid and uneventful, no sequelae could be detected by clinical inspection.

Results

[illegible] results are given as mean values derived from series of 6 consecutive expts. Within each [illegible] consistent responses were obtained. The results obtained from analysis of samples of [illegible] venous blood are shown in Fig. 2, the values which were obtained in series *b* and [illegible] indistinguishable from those obtained in series *a*. The infusion of GU-solution from [illegible] to 11.10 lowered the osmolality of plasma by 3–4% and produced a slightly larger [illegible] decrease in the concentration of Na. The mean concentration of K exhibited [illegible] variations. Apparently the values obtained in the morning in series *d* and [illegible] are [illegible] than those of series *a–c*. The reason for this is not clear. Separate expts. in conscious [illegible] showed that application of venous stasis (cuff pressure of 70–90 mmHg for 5 min) was [illegible] followed by any changes in the concentration of potassium in plasma, therefore, the [illegible] discrepancy cannot be ascribed to different degrees of venous stasis during blood [illegible]. From Fig. 2 can be seen that once the hydration was established the concentra[illegible] of Na and K and the osmolality did not change throughout the control experiments. [illegible] infusions of hyper-osmolar NaCl solution (series *d* and [illegible]) caused an increase in plasma [illegible] and in the concentration of sodium in plasma as well as a decrease in the con[illegible] of K and a delay of the steady increase in hematocrit which was seen in series [illegible]. The direction and magnitude of these responses are compatible with the assumption

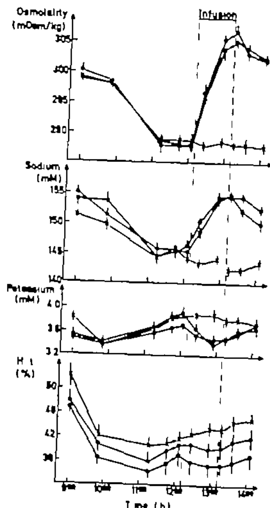


Fig. 2 Hematocrit, concentrations of Na+ nd plasma, and plasma osmolality during differen of expts. Control expts ( ) in which no test w was infused Intra enous (O) nd bilateral carot d (●) infusions of hyper-osmolar sodium ride solutions, 90 μmol/(kg b wt. min) and 45 (kg b wt. artery min), respect ely The animal anesthetized at 09 00–09.30 a.m. and infusion solution was performed during the period indi Values are mean ± S.E., n = 6.

that infusion of the hyper-osmolar sodium chloride solution caused a shift of water fron intracellular to the extracellular fluid space.

Water excretion and osmolar clearance

Diuresis and osmolar clearance in the course of the series of experiments are shown in Fi Free water clearance is equal to the vertical distance between the two graphs shown in part of the figure, it is positive when diuresis is greater than osmolar clearance The infus of vasopressin caused a decrease in diuresis and in free water clearance (Fig. 3 b and and these effects were apparently dependent on the rate at which vasopressin was infu

From Fig. 3 d and 4 it is seen that the immediate response to i v infusion of hyper-osmo NaCl solution was biphasic. During the first half of the infusion the osmolar clearance unchanged whereas the diuresis (Fig. 3 d) and the free water clearance (Fig. 4) increas during the last part of the infusion period the osmolar clearance showed a substantial crease while the diuresis and the free water clearance declined Negative free water clearar (Fig. 4 and intersection of graphs in Fig. 3 d and 3 e) appeared approximately 10 min af termination of the infusion.

From Fig. 3 d, 3 e and 4 it seems justified to conclude that the renal response to infusi

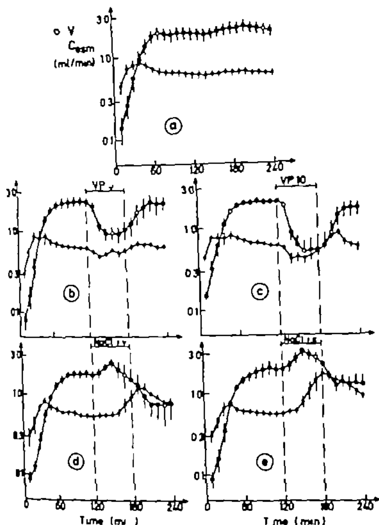


Fig. 3. Diuresis (O) and osmolar clearance (●) as function of time during 5 series of expts: *a*, control expts, hydration at constant rate in period 0–60 min followed by sample maintenance in period 60–240 min; *b*, infusion of vasopressin at rate of 5 μU/(kg b. wt. min); *c*, infusion of vasopressin at rate of 10 μU/(kg b. wt. min); *d*, intravenous infusion of hyper-osmolar sodium chloride solution at rate of 90 μmol/(kg b. wt. min); *e*, bilateral infusion of hyper-osmolar sodium chloride solution at rate of 45 μmol/(kg b. wt.·artery·min). Periods of infusion of vasopressin or sodium chloride are indicated by dashed lines. Time 0 corresponds to 10.10 a.m. Note the logarithmic spacing of the vertical scale. Values are mean ± S.E., n = 6.

into the common carotid arteries of a strongly hyper-osmolar sodium chloride solution was not significantly different from the response to i.v. infusion. The effects of NaCl infusion differed from those of vasopressin administration by (*i*) an initial increase in free water clearance, (*ii*) appearance—within the period of infusion—of an increase in osmolar clearance, and concurrent with the latter (*iii*) a considerable increase in the rate of excretion of sodium.

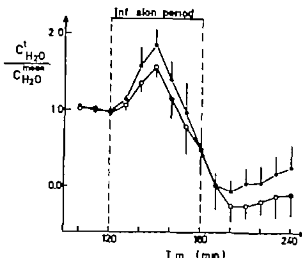


Fig. 4 Changes in free water clearance du
i (○) and intracarotid (●) infusion
hyper-osmolar sodium chloride solution
rate of 90 μmol/(kg b.w.t. min). In each e
the free water clearance in each of the
14 periods of collection ($C^{i}_{H_2O}$) was divided
the mean of the three clearances preceding
infusion i.e $C^{mean}_{H_2O}$ – ($C^{10}_{H_2O}$ + $C^{11}_{H_2O}$ + $C^{12}_{H_2O}$
Time 0 corresponds to 10 10 m. The re
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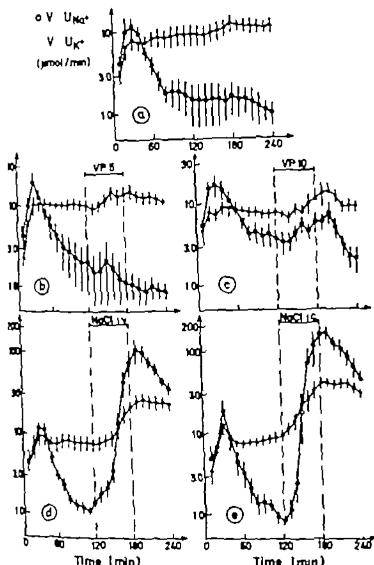
The rate of excretion of sodium

The rates of excretion of sodium and potassium during the series of expts. are shown Fig. 5 panel *a* shows that the course of these rates in the control series was similar to t observed earlier (Bie 1976 b, 1976 c) *i e* an apparent increase during hydration followed a decrease in the rate of excretion of sodium whereas the rates of excretion of potass throughout the expts. remained at an elevated level when compared to prehydration valu This pattern was not changed by administration of vasopressin (Fig. 5 b and 5 c). Dur the infusion of hyper-osmolar sodium chloride solution the rate of excretion of sodi increased from about 1 0 μmol/min to 100–150 μmol/min (Fig. 5 d and 5 e) During t infusions no change could be measured in either the clearance of p-aminohippuric acid in glomerular filtration rate, therefore, fractional sodium excretion increased concomit with the absolute rate of excretion *i.e* the rate of excretion of sodium increased from le than 3 10^{-4} to 0.02–0 04 times the rate of filtration. The rate of excretion of potassiu showed a modest increase and the ratio between the concentrations of sodium and potassiu in urine changed from approximately 0 1 before infusion to a maximum of 4–5 just after t infusion

The results thus show that the rates of excretion of Na and K increased 100-fold an 3-fold, respectively when plasma osmolality and sodium concentration was normalized b infusion of hyper-osmolar sodium chloride solution following after sodium-free volum expansion and that apparently identical alterations occurred irrespective of the mode o administration.

Central venous pressure

The results of the measurements of the central venous pressure are shown in Fig. 6. Th values obtained in series *b* and *c* (infusions of vasopressin) were not significantly differen from—or were lower than—those obtained in control expts. From Fig. 6 is seen that th hydration procedure caused a lasting increase in central venous pressure of about 1.0 cm o water It also appears that the i v infusion of hyper-osmolar sodium chloride solution wa not associated with values of central venous pressure different from t



...e rates of excretion of sodium (O) and potassium (●) as function of time during 5 series of expts. ...Fig. 3

...experiments. Intracarotid infusions of hyper-osmolar saline was associated with ...f mean central venous pressure which were approximately 1.0 cm of water above ...tained in the control series.

Discussion

...ment expts. were designed in order to re-examine the osmoreceptor hypothesis ...r 1947). According to this hypothesis the covariation of the osmolality of the blood

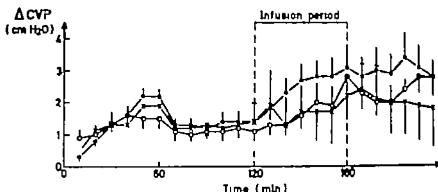


Fig. 6. Changes in central venous pressure as function of time in control expts. ( ) and in 2 series of expts in which hyper-osmolar NaCl solution was infused i.v (O) or i to the carotid arteries (●). Hydration performed in the period 0–60 min. Pressure = 0 corresponds to the value obtained at time = −5 min, time 0 at 10 10 a.m. Results are mean ± S.E., n = 6.

and the rate of secretion of vasopressin is mediated by an "osmoreceptor" situated *within the area supplied by the common carotid arteries* The results of the present expts. support the view that diuresis and the rate of excretion of "free water" may be controlled by an "osmoreceptor" but they are inconsistent with the hypothesis with regard to the location of the receptor This statement rests upon several assumptions concerning the concentration of vasopressin in plasma during the expts. and the osmolality to which the anterior part of the hypothalamus was exposed during the intracarotid infusions. The possible influence of the anesthetic and the volume of the hydration fluid on the rate of secretion of vasopressin has been dealt with earlier (Bie 1976 a, 1976 b), in addition, the validity of the conclusions drawn from the present results may be questioned (i) if the infusions of hyper-osmolar sodium chloride solutions were not performed during a water diuresis (defined as a condition characterized by a low rate of secretion of vasopressin), (ii) if the renal antidiuretic response to the infusion of the NaCl solutions was elicited exclusively by factors other than an increase in the concentration of vasopressin in plasma, and (iii) if the regional hyper osmolality created by the bilateral infusions into the common carotid arteries did not include the anterior hypothalamic area in which the "osmoreceptors" are said to be localized (Jewell and Verney 1957 Woods, Bard and Bleier 1966)

(i) In the present expts. solutions containing NaCl or vasopressin were infused into animals under circumstances apparently fulfilling all criteria of a water diuresis. The diuresis measured approximately 0.2 ml/(kg b wt. min), urine osmolality was between 75 and 120 mOsm/kg of water the rate of excretion of sodium was low and the urine did not contain abnormal amounts of glucose. The concentration of urea in the GU-solution was roughly equivalent to the concentration in the urine during the experiments and there is no reason to expect, therefore, that the infusion of the GU-solution caused an increase in the concentration of urea in plasma. It thus appears that the diuresis increased as a result of changes different from those eliciting an osmotic diuresis. The sensitivity to exogenous vasopressin strengthens the impression of a water diuresis. Graded antidiuretic responses were obtained by infusions of 55 and 110 μU/min to dogs weighing approximately 11 kg. From expts. on conscious dogs with diabetes insipidus weighing 7–12 kg Shannon (1942) concluded that graded antidiuresis

obtained by infusion of 0.001–0.005 units per hour corresponding to 17–83 μU/min. rs ago it was concluded that dogs in chloralose anesthesia excrete a water load as rapid onscious animals (Theobald 1934). Thus it seems justified to assume that the condition ng which vasopressin or hyper-osmolar solutions were infused was that of a water esis elicited by a considerable decrease in the rate of secretion of vasopressin.

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iii) Calculations of the intensity of the osmotic stimulus to which the anterior hypothalamus as exposed during intracarotid hyper-osmolar stimulation require information cerning the degree of mixing of the infusate with carotid blood and the blood flow ough the common carotid arteries. When slow intracarotid infusions are used in studies osmotic stimulation it is generally assumed that complete mixing occurs within the mmon carotid artery (Bie 1976 a, Eriksson, Fernández and Olsson 1971 Verney 1947 ldens, Clarke and Minton 1956) Consistent responses in series of consecutive experiments in different animals support the validity of this assumption. It seems unlikely that large part of the infusat in all expts would by-pass the origins of the internal carotid teries and the rami anastomotici of the maxillary arteries through which—at least in the g—the circulus arteriosus is supplied (Gillilan 1976). It therefore seems reasonable to culate—as Verney (1947) did—an approximate increase in the osmolality of the blood flowing the hypothalamus from the rate of infusion of NaCl and an estimate of the blood ow through the common carotid arteries.

It thus appears that none of the said three reservations apply to the present studies. The fect of the intracarotid infusion on the osmolality of the carotid blood depends on the lood flow and the rate of infusion. Assuming a flow through a common carotid artery of ml (kg b wt. min) it can be calculated (Bie 1976 a) that the intracarotid infusions increased e osmolality by some 12 mOsm/kg of water i.e. In series c the osmolality of the blood ing the carotid arteries exceeded the values shown in Fig. 2 (upper panel) by this amount hroughout the infusions. By considering the slopes of these graphs during infusion it is en that at the very beginning of the intracarotid infusions the osmolality of the carotid ood increased to prehydration values which during i.v. infusion were not obtained until ome 25 min later. It also appears that at the end of the i.v. and the intracarotid infusions he carotid osmolality was approximately 305 and 320 mOsm/kg, respectively. Despite these elatively large differences the antidiuretic responses in the two series of experiments were ndistinguishable.

Previous work (Bie 1976 a) did not include results which supported any osmoreceptor theory therefore, from these negative expts. it was concluded that the validity of the hypothesis of Verney appeared questionable. The present results are in accordance with the view that the rate of secretion of vasopressin is accurately controlled by a receptor mechanism

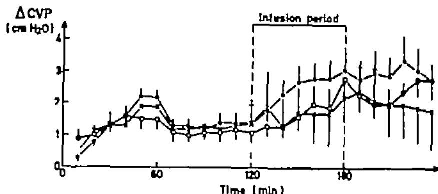


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The rate of excretion of sodium

The present work shows—in accordance with previous results (Bie 1976 b)—that hypo-osmolar volume expansion is associated with a decrease in the rate of excretion of sodium. The present expts. with vasopressin infusions show that this hormone may cause a decrease in diuresis and an increase in urine osmolality without a concomitant increase in the rate of excretion of sodium. It has been shown repeatedly that vasopressin is capable of enhancing this variable (*e.g.* Buckalew and Dimond 1976, Humphreys, Friedler and Earley 1970), however the phenomenon has been demonstrated only by infusion of vasopressin at rates which were larger than any reasonable estimate of the maximal rate of secretion from the neurohypophysis. It is uncertain whether naturally occurring concentrations of vasopressin may induce an increase in the rate of excretion of sodium.

The changes in absolute as well as fractional sodium excretion during infusion of hyper-osmolar sodium chloride solutions seem massive and the chain of events between the infusions and the increase in the rate of excretion of sodium deserves further investigation.

The author is grateful to Mrs K. Bülow for her skilled technical assistance. A densitometer was placed at our disposal by Dr S. Lundh, The Protein Laboratory, University of Copenhagen, whose cooperation is greatly appreciated. Parts of the expenses were defrayed by grants from the Danish Medical Research Council (J. nr. 512 7140) and Nordisk Insulinfond. The author was supported by a grant from J. and H. Weimann's legacy.

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est that the cellular transmembrane potential changes are more sensitive in revealing the effects of shock at the cellular level and precede significant interstitial electrolyte changes.

Various types of hypoperfusion shock conditions will result in a heterogeneous distribution of the microvascular flow (see e.g. Nicoll and Frayser 1967) and severely interfere with cellular metabolic function (Baue *et al.* 1974). Assuming that transmembrane potential is sensitive in revealing the cellular metabolic consequences of hypoxia in single skeletal muscle fibers, then the extent of heterogeneity in distribution of nutritive flow should be demonstrable from such transmembrane potential measurements.

In the present study a model with exteriorization of the intestine, resulting in severe shock (Bergentz *et al.* 1969), was used. The cellular membrane potential changes in the progress of shock was registered. Comparison was made to the transmembrane potential reaction to complete ischemia.

Material and Methods

Mongrel dogs (n = 18), weighing 20–28 kg, were used. After atropine 0.3–0.5 mg and diazepam 5 mg i.v., anesthesia was induced with ketamine 2–3 mg/kg b.w. The dogs were intubated after muscular relaxation with pancuronium bromide and ventilated with air to keep a [illegible] P CO_2 of 4.0–4.5 kPa. Anesthesia was maintained throughout the expt. by the use of slow ketamine infusion and additional doses of pancuronium bromide.

One of the carotid arteries was cannulated for continuous registration of blood pressure, for repeated blood gas analyses as respiratory control, and for hematocrit determinations. The preparation was allowed to stabilize for approximately 1 h before the expt.

The following groups of experimental animals were studied.

Group I. Control animals, subjected to anesthesia, insertion of catheters and sampling procedures. Sham operation consisting of laparotomy without exteriorization of the intestine was performed.

Group II. Shock was induced by exteriorization of the small intestine with the addition of slight strangulation as previously described (Bergentz *et al.* 1969). After 3 h the intestine was replaced and the abdominal wall closed. The dogs were studied for an additional period of 2–3 h.

Group III. Tourniquet occlusion of one of the hind limbs was produced by the application of a narrow blood pressure cuff at 300 mmHg after removal of the blood with an Esmarch bandage. The tourniquet was kept in place for 3 h and then released. A 3-h post tourniquet time period was studied. The same sham laparotomy as in Group I was also performed.

Modified Ling Gerard microelectrodes were used for the transmembrane potential registrations (Ling and Gerard 1949, Cunningham *et al.* 1969). The microelectrodes were drawn from borosilicate capillary tubing (outer diameter 1.85–1.95 mm) using a mechanical puller. The tip of the electrode was gently broken under microscope in order to give a sharp tip of 1–2 μm. The microelectrodes were filled with 3 M KCl. Electrodes with tip resistances of 4–10 megohms were used. The tip potentials were usually 1–3 mV. A small (outer diameter 2 mm) Ag-AgCl electrode the tip of which was inserted subcutaneously served as a reference electrode. The microelectrode was connected to a high input impedance electrometer amplifier and potentiometric recorder (LKB 6520).

A small incision of approximately 1 cm^2 was made down to the fascia of the gracilis muscle. The surface was covered with mineral oil and a small slit was gently performed in the fascia to expose the muscle. With the aid of a micromanipulator the microelectrode was advanced through the oil until contact was made with the tissue fluid. This was considered the zero point. The microelectrode was slowly advanced through the muscle, and the transmembrane potential was registrated for the penetrated muscle cells. The transmembrane potential of the most superficial cells was always low. On passing through successive layers of muscle cells, there was a gradual increase in transmembrane potential until, at a depth of about 1–2 mm, a fairly stable plateau was reached. Registrations from these superficial cells were not included in the material, as they probably represented injured cells (Cunningham *et al.* 1971 a). A penetration was considered successful

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Transmembrane Potential Measurements as an Indicator of Heterogeneous Distribution of Nutritive Blood Flow in Skeletal Muscle during Shock

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Abstract

HALJAMÄE, H E. JENNISCHE and A MEDEGÅRD *Transmembrane potential measuremen as an indicator of heterogenous distribution of nutritive blood flow in skeletal musc during shock* Acta physiol scand 1977 *101* 458–464

The heterogeneity of nutritive skeletal muscle blood flow during shock was studied in terms of cellul transmembrane potential variations. Shock was induced in dogs by exteriorization of the small intestine f 3 h, whereafter the intestine was replaced and the d gs were studied for another 3–4 h. Transmembra potentials of single skeletal muscle cells were recorded by the use of modified Ling-Gerard microelectrode The mean resting transmembrane potential in control animals was −90 4 mV±0.7 The variation in tran membrane potential between adjacent fibers was small. During shock there was a significa t decrease in th mean transmembrane potential and at the same time there was an increasing variation of up t 29 mV in th values between adjacent fibers. To exclude metabolic differences between red and white f bers as the mai reason fo this increasing heterogeneity of transmembrane potentials of adjacent fibers, complete tourniquet ischemia was also studied. The total tissue ischemia resulted in a marked reduction of the transmembran potential in all cells with o ly small differences between adjacent f bers After release of the tourniquet marked spread of resting membrane potentials, with variations of 17 25 mV between adjacent fiber occurred This variation was similar to that bserved during shock It is concluded that repeated tran membrane potential registrations may reveal the variable state of adjacent cells subjected t a heterogeneou nutritive blood flow in shock.

Key words. Skeletal muscle, hypoperfusion shock, transmembrane potentials, utritive blood flow

Transmembrane potential measurements have been used as a possible method for direct monitoring of the pathophysiological changes occurring at the cellular level in skeletal muscle in shock (Campion *et al* 1969 Cunningham *et al* 1971 b Shires *et al* 1972, Trunkey *et al* 1973) ischemia (Arango *et al* 1976), and in severely ill patients (Cunningham *et al* 1971 a). The changes in transmembrane potential seem mainly due to "hidden" local changes in the electrolyte distribution across the cell membrane during shock as shown by direct sampling and analyses of the electrolyte content of interstitial fluid (Hagberg *et al* 1968 Haljamäe 1970 a, 1970 b) and of single cells (Haljamäe 1970 c) Cunningham *et al* (1971 b) found, how-

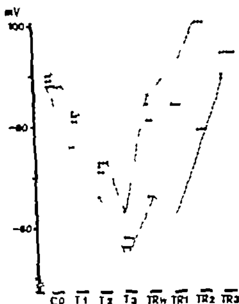


Fig. 3 Ten consecutive transmembrane potential registrations from adjacent cells before (CO), after 1, 2, and 3 h of complete tourniquet ischemia (T), and after ½, 1, and 3 h following release of the tourniquet (TR), are exemplified for one animal. Dotted lines indicate range limits.

Transmembrane potential registrations from control animals during prolonged anesthesia for up to 6 h is exemplified in Fig. 1. Ten consecutive registrations at each time period are plotted. There were no significant changes in mean transmembrane potential during the control period. The variation in transmembrane potential between adjacent cells was very small, thus all cells measured maintained a transmembrane potential close to 90 mV throughout the control period.

During shock there was a progressive decrease in mean transmembrane potential, and in addition there was an increasing variation in transmembrane potential between adjacent cells. This is exemplified in Fig. 2.

In Fig. 3 the effect of complete ischemia on the transmembrane potential is exemplified. Prolonged ischemia resulted in a significant decrease in the mean transmembrane potential, the decrease was more pronounced than that seen after corresponding time period of shock. The values of adjacent muscle fibers were at a similar level, i.e. the variation between adjacent cells was small and comparable to that during control conditions. After the release of the tourniquet there was a continuous increase in mean transmembrane potential. At the same time, however, there was a markedly increased variation in transmembrane potential between adjacent cells. After about 3 h transmembrane potentials returned to preischemic values, with small variation between adjacent fibers.

In Table I the data from 6 dogs in each experimental group are presented. Each individual value in the Table, represents the mean of 10 consecutive successful registrations from adjacent cells ± S.D. at one penetration into the tissue. As can be seen from the Table, early (1-2 h) as well as late (4-5 h) shock resulted in a significantly (p 0.01) decreased transmembrane potential. Tourniquet ischemia caused similar (p 0.01) decrease in mean transmembrane potential.

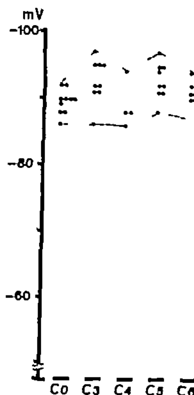


Fig. 1

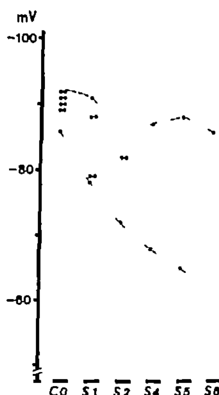


Fig. 2

Fig. 1 Separate transmembrane potential values of ten consecutive registrations from adjacent cells various times in one control experiment. C0–C6 indicate hours after start of the experiment. Dotted lines indicate range limits.

Fig. 2. Ten consecutive transmembrane potential registrations from adjacent cells exemplified before (C and after 1 to 6 h of intestinal exteriorization shock (S1–S6) in one animal. Dotted lines indicate range limits.

If it was possible to keep the tip of the electrode within the cell for some seconds, and if the registration during this time remained at a rather stable niveau. Measurements were made at regular intervals and every time a fresh muscle area was exposed. Repeated registrations were made with several electrodes.

Wilcoxon's two-sample rank test (the Mann-Whitney test) was used for the statistical analysis.

Results

The intestinal exteriorization with slight stasis (Group II) resulted in a progressive decrease in blood pressure. The initial mean value was 141 mmHg, S.E. ± 5. After 1 h a reduction to 112 ± 6, after 2 h to 96 ± 12 and after 3 h to 72 ± 11 mmHg was observed. The replacement of the intestine and closure of the abdomen resulted in a transient slight increase to 78 ± 10 mmHg, followed by a progressive circulatory deterioration and a blood pressure of only 35 ± 11 mmHg 3 h later.

Dogs subjected to tourniquet ischemia (Group III) had stable pulse and blood pressure except a slight transient decrease in blood pressure at the release of the tourniquet.

In control animals (Group I) pulse and blood pressure were mainly unchanged during the 6 to 7 h studied.

TABLE I Transmembrane potential registrations from skeletal muscle f bers during initial and 3 l control conditions during early (1 2 h) and late (4–5 h) intestinal exteriorization shock 1 2 h of complete tourniquet ischemia and 1–2 h after release of the tourniquet. Each indivi value represents the mean of ten consecutive successful registrations from adjacent cells ± S at one penetration into the tissue. 6 animals were studied in each group.

	I tial values		Co trols 2 3 h		Early shock 1 2 h		Late shock 4–5 h		Tourniquet 1 2 h		After releas 1 2 h	
	−mV	±S.D	−mV	±S D	−mV	±S.D	−mV	±S.D	−mV	±S.D	−mV	±S
	88.9	1	89 6	2	83 6	4	78.3	4	79 9	3	77.4	6
	89.5	2	92.2	3	79 0	5	77 7	8	65 8		88 6	9
	89 0	3	93 7	3	85 6	6	70.3	7	79.2	3	73 6	6
	89 7	2	93.2	3	84.0	7	72.0	7	70.7	3	82.7	6
	91 9	3	84 1	1	82.8	6	75 1	8	63.6	3	88 6	6
	93 1	3	88 1	2	81.3	5	65 1	9	66.7	3	89.2	7
Mean	90 4		90.2		82.7		73 1		71.0		83 4	

p < 0 01 *vs* control values.

In each animal, however the individual values show that the shock induced decreas mean transmembrane potential, is combined with an increased variation between adja cells. This variation increased throughout the shock period (*cf* Fig. 2). If the indivic S D values are taken as a measure of this variation, than significant (p < 0.01) differen were at hand between control animals and shock animals. Tourniquet ischemia, on the ot hand, did not result in such an increased variation After the release of the tourniquet increased variation (p < 0 01 *vs* controls) similar to that during shock occurred.

In control animals no changes in either the mean transmembrane potential or in variation between adjacent fibers were observed

Discussion

The technique used for the transmembrane potential registrations was highly reproduci and the obtained control values of − 88.9 – − 93 1 mV for resting skeletal muscle potent are in agreement with previous reports on mammalian skeletal muscle. The decrease in mean transmembrane potential in the course of the shock *i e* exteriorization of the sm *intestine, was also similar to that previously reported for hemorrhagic* shock in rats, d and primates (Campion *et al* 1969 Cunningham *et al* 1971 b Shires *et al* 1972, Trunl *et al* 1973 Arango *et al* 1976).

There seems to be a direct correlation between the extent of cellular metabolic impairm and the mean cellular transmembrane potential *reduction during shock (Jennische et* 1977) It may therefore be assumed that such disturbances in cellular phosphagen and me bolite levels result in an interference with the cellular membrane electrolyte transport and increase in cellular sodium and loss of intracellular potassium (Haljamäe 1970 b, Shi *et al* 197?)

The increased variation in transmembrane potential between adjacent cells during sho may be considered as an indication of a marked difference in the metabolic deteriorati

een adjacent cells and of an uneven distribution of nutritive blood flow in the muscle. It ell-known that the perfusion in the terminal vascular bed is rather heterogeneous (see el and Fraser 1967). However during normal resting conditions vasomotion provides ient nutritive flow to each cell. Direct intravital microscopic studies of the flow pattern e microvessels of skeletal muscle during shock has given direct evidence for a progressive tion of microvessels leading to an extensive "functional shunting" at the capillary level tsson and Lisander 1972). The studies of Appelgren (1972) of perfusion and diffusion in ck by the use of clearance of ¹³³Xe for tissue perfusion and ¹²⁵I for tissue diffusion also ate heterogeneity of nutritive flow during shock.

has previously been reported that there is a difference in the resting transmembrane ntials between red and white muscles of the rat (Yonemura 1967) and Goodgold and rich (1966) have suggested the existance of two families of muscle fibers in the human s abdominis muscle, each having different transmembrane potential. No such grouping subgroups of control resting membrane potentials could be seen in the muscle studied in present expts. The tourniquet ischemia situation was used as a model to exclude the ability of such a different sensitivity of various groups of fibers to the hypoxia as an lanation for the observed spread of transmembrane potentials during shock. If such a rent sensitivity of various fibers were a main reason, then one would expect a similar ogeneity of transmembrane potentials in the early phase of tissue anoxia after the lication of the tourniquet. No such spread of the values was observed and the distribu- early after the tourniquet application was similar to that during prolonged tourniquet d diffusion may be assumed to equal out metabolite discrepancies between adjacent cells. After the release of the tourniquet, during the first 2 h of recirculation, there was, however ilar spread of transmembrane potentials as observed during shock. This finding is well ement with the observations of Romanus *et al.* (1977) on the microcirculatory events f prolonged pressure-induced ischemia in the hamster cheek pouch. The release of the ure after 3–4 h was reported to result in an initial low number of open microvessels, the ber of which slowly increased to about 50% of controls after 2 h of recirculation.

It is therefore concluded that transmembrane potential registrations may reveal functional erences between adjacent cells, which probably are caused by a heterogeneous distribution he nutritive blood flow to the cells.

This work was supported by grants from the Swedish Medical Research Council (Projects B76-17X-00127 17X-00660, B77-12X-04964) and Tore Nilssons Fund for Medical Research.

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Acta physiol. scand. 1977 101 465–475

From the Department of Physiology, Karolinska Institutet, Stockholm, Sweden

Electrophysiological Membrane Properties of the Frog Muscle Fibre: Effects of Detergents in the Triton Series

By

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Abstract

Rydqvist B. *Electrophysiological membrane properties of the frog muscle fibre. Effects of detergents in the Triton series.* Acta physiol. scand. 1977 *101* 465–475

The effect of the nonionic detergent Triton X 100 on the membrane properties of frog sartorius muscle fibres was studied using intracellular microelectrode technique. The effect of Triton X-100 was compared with that of Triton X-45 and Triton N-101. At 40 µM Triton X 100 had little effect on the specific membrane resistance (R_m), membrane capacitance (C_m) and the resting membrane potential (E_r) while the action potential (AP) was markedly reduced. At 160 µM AP was completely abolished and E_r was diminished linearly with time. The effect on the maximum rate of rise (V_A) of the AP was dose-dependent and the apparent dissociation constant ($K_{D\,app}$) and K_D were found to be about 40 µM. The Hill coefficient was 1.1 indicating deviation from a first order reaction. The effect of Triton X 100 on E_r may be accounted for by a reduction of the Na–K pumping activity. The effect of Triton X-100 on the AP is suggested to be due to perturbation of a protein-system either by a drug–receptor interaction involving two or more sites, or unspecific binding of Triton X-100 to hydrophobic loci on the protein.

The nonionic detergents have been extensively used in the solubilization of membranes (Helenius and Simons 1975) and isolation of membrane-bound proteins, for example the cholinergic receptor protein (Miledi and Potter 1971, Meunier, Olsen and Changeux 1972, cf. Changeux *et al.* 1976). A number of nonionic detergents are known to cause a reduction of the contractile response of muscle to nerve stimulation (Soehring, Dees and Sta 1952). These detergents (e.g. alkyl-polyglycolethers) seem to have similar effects as local anesthetics (Dees and Soehring 1952, Dittmann 1973) and produce anesthesia of the cornea (Zipf and Dittmann 1964, Harris, Kahanowicz and Shilomyo 1975). Furthermore, as shown by Brisson, Devaux and Changeux (1975), Triton X 100 reversibly blocks the response of *Electrophorus* electroplaques to carbamylcholine without affecting the resting membrane potential.

The nonionic detergents in the Triton series belong to a class of lipids called amphiphiles, that is, substances with a polar (hydrophilic) and an apolar (hydrophobic) part. Work on homologous alkyl-polyglycol-ethers (nonionic detergents similar to the *Tritons*) has yielded some insight into the relative importance of the hydrophobic and hydrophilic parts of

these molecules with regard to their blocking potency on the nerve–muscle-preparation (Zipf and Dittmann 1964 Dittmann 1973) However very little is still known about how detergents affect the membrane properties of muscle and nerve fibres.

The present experiments were carried out in order to obtain information about the influence of some Triton detergents on the active and passive electrophysiological properties of the frog muscle cell membrane. Classical drug–receptor kinetics have been used to describe some of the observed effects of Triton X 100

Methods

The experiments were performed on the isolated frog sartorius muscle (*Rana temporaria*) The muscle as mounted in a bath with its deep surface upward and stretched to 110–120% of its resting length The preparation was maintained at room temperature (about 23°C) The Ringer solution had the following composition NaCl 115 mM KCl 2.5 mM $CaCl_2$ 1.8 mM, Na_2HPO 2.15 mM and NaH_2PO 0.85 mM pH 7.2 (Adrian 1956) The experimental procedure was the same as described in an earlier paper (Boéthius and Rydqvist 1977).

Membrane potentials were recorded with glass capillary microelectrodes filled with 3 M KCl, resistance about 10 MΩ and tip potential of less than 5 mV Microelectrodes used to inject current were filled with 2 M K-citrate, resistance bout 10 MΩ. Care was taken only to impale surface fibres. In order to avoid penetrating the same fibre twice the electrodes were systematically moved across the muscle in one direction only The current electrode was inserted about 25–50 μm from the voltage electrode and before the action potential was elicited the membrane was locally polarized to − 90 mV in order to obtain uniform activation of all fibres.

Membrane potential changes were displayed on an oscilloscope coupled to the microelectrode through a high input mpedance amplifier In addition the resting membrane potential was monitored on digital voltmeter The current passed through the fibre was displayed on the oscilloscope as the voltage drop across the feed-ba k resistor in an operational amplifier circuit used to hold the bath at virtual ground potential. The rate of rise of the action potential was obtained by differentiating the action potential electrically using a Miller differentiator and then displaying the utput from the differentiator on the oscilloscope together with potential changes.

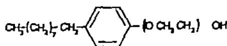
Membrane constants were obtained by rectangular pulse analysis for the case of an infinite cable (Hodgkin and Rushton 1946, Fatt and Katz 1951 Hodgkin and N kajima 1972, see also Boéthius and Rydqvist 1977) and the specific membrane resistance (R_m) was calculated assuming the internal resistance of the fibre (R_i) to be 200 Ωcm (Fatt 1964).

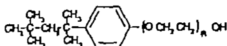
Triton X-45 (TX-45), Triton X 100 (TX 100) and Triton N 101 (TN-101) were achieved from Rohm & Haas Co (Sweden) and used without further purification. TX-45 and TX 100 belo g to series of homologous polyoxyethylene p–t-octylphenols with the structural f rmula shown in Fig 1 (Triton surface active agents, the nonionic octylphenoxyethanol (OPE) series. CS-40 I/cf Rohm & Haas Co, Philadelphia) TX-45 has a *n*-value between 4 and 5 and molecular weight of 426. TX 100 has a *n*-value between 9 and 10 and a molecula weight of 628 (Rohm & Haas Surfactants, Handbook of Physical Properties CS-Ola, Rohm & Haas Co Philadelphia). According to Simons *et al.* (1973) the mean value for TX 100 is 9 8 The hydrophilic lipophilic balance number (HLB) is 10 4 for TX-45 and 13.5 for TX 100. None of these substances are homogeneous in thei hydrophilic moiety being distributed according t Poisson (Schick 1967). Also the hydrocarbon part of the molecules is inhomogeneous to a small extent.

TN 101 is a polyoxyethylene nonylphenol (F g. 1) with between 9 and 10 and HLB number of 13 4 (Rohm & Haas Surfactants, Handbook of Physical Properties CS-Ola, Rohm & Haas Co, Philadelphia) The detergents were solved n the Ringer solution to the actual concentrations.

Results

TX-45 TX 100 and TN 101 produced no gross morphological changes as observed in the dissecting microscope and no spontaneous contractions could be observed for the con-



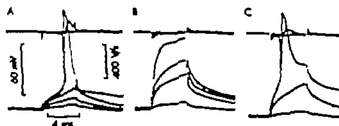


Structural formula for the detergents in the X and Triton N series. n indicates the number [illegible] units

[illegible]tions used in the present study (i.e. 10–320 μM). The effects of TX 100 were primarily [illegible]ed and TX-45 and TN-101 were later applied at a few concentrations and the effects [illegible]ced by these detergents compared to those of TX 100. The resting membrane properties [illegible] studied using the resting membrane potential (E_r), the specific membrane resistance [illegible] and the membrane capacitance (C_m). The maximum rate of rise (V_A) was used to analyze [illegible]ges in the action potential (AP). This parameter was chosen as it is a good approxima[illegible] for the sodium current (I_{Na}) underlying the muscle action potential.

[illegible]cts of Triton X 100

[illegible] general effect of TX 100 on the muscle action potential is illustrated in Fig. 2 for three [illegible]esentative fibres at different times. Record (A) shows the AP in normal Ringer solution. [illegible]r 20 min in 160 μM TX 100 the muscle fibre AP is completely abolished (B) and the [illegible]ntial changes produced by the current pulses reflect the passive properties of the [illegible]mbrane only. After 20 min the muscle was returned to normal Ringer solution and about [illegible]min later an almost normal AP could be obtained (C). However, the differentiated signal [illegible]per trace) clearly shows that the recovery was not complete. The maximum rate of rise [illegible]) after the muscle had recovered in normal Ringer solution is about 400 V/s in this fibre [illegible]ich should be compared with the control value of about 600 V/s. This indicates that the [illegible]ct of the detergent at this concentration (160 μM) is not fully reversible although the [illegible]scle was allowed to recover in normal Ringer solution for 75 min. This is also illustrated



[illegible] 2. Records from frog muscle fibres showing the change in action potential (lower trace) and rate of [illegible] (upper trace) produced by TX 100 (160 μM). T[illegible] obtain uniform activation conditions, the fibres were [illegible]perpolarized to [illegible]90 mV for [illegible] bout 30 s before eliciting an action potential. A. Normal Ringer solution. [illegible] After 20 min in TX-100, 160μM. C. After 75 min in normal Ringer solution.

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The present experiments were carried out in order to obtain information about the influence of some Triton detergents on the active and passive electrophysiological properties of the frog muscle cell membrane. Classical drug-receptor kinetics have been used to describe some of the observed effects of Triton X 100

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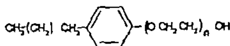
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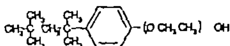
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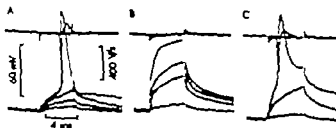


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Triton X 100

...al effect of TX 100 on the muscle action potential is illustrated in Fig. 2 for three ...tive fibres at different times. Record (A) shows the AP in normal Ringer solution. ...min in 160 μM TX 100 the muscle fibre AP is completely abolished (B) and the ...changes produced by the current pulses reflect the passive properties of the ...only. After 20 min the muscle was returned to normal Ringer solution and about ...ter an almost normal AP could be obtained (C). However the differentiated signal ...ce) clearly shows that the recovery was not complete. The maximum rate of rise ...the muscle had recovered in normal Ringer solution is about 400 V/s in this fibre ...uld be compared with the control value of about 600 V/s. This indicates that the ...the detergent at this concentration (160 μM) is not fully reversible although the ...as allowed to recover in normal Ringer solution for 75 min. This is also illustrated



...ords from frog muscle fibres showing the change in action potential (lower trace) and rate of ...r trace) produced by TX 100 (160 μM). T... obtain uniform activation conditions, the fibres were ...rised to ... 90 mV for about 30 ... before eliciting an action potential. A Normal Ringer solution. ...0 min in TX 100, 160μM. C After 75 min in normal Ringer solution.

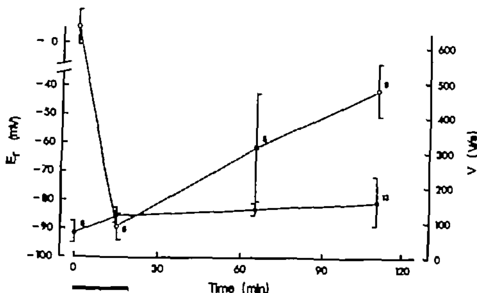
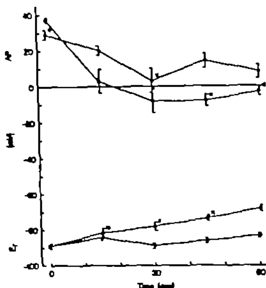


Fig. 3 Resting membrane potential (E_r) and maximum rate of rise (V_A) vs time for muscle exposed to 160 μM Triton X 100 for 20 min (bar) and allowed to recover in normal Ringer solution for 100 min. The mean E_r and V_A are plotted in closed and open circles respectively. The bars are the standard deviation (S.D.) of the mean. The number of fibres used to estimate mean and S.D. are indicated by figures at each point. The mean and S.D. for 15 min was calculated from measurements between 10 and 20 min, the mean and S.D. for 67 min was calculated from measurements between 64 and 70 min and the mean and S.D. for 112 min was calculated from measurements between 104 to 120 min.

In Fig. 3 which shows result from several fibres of a muscle which was treated at 160 μM for 20 min and allowed to recover in normal Ringer solution for 100 min. As can be seen the resting membrane potential (E_r) is only slightly affected during the exposure the fibres depolarised 6 mV (from −91.5 to −85.5 mV after 15 min) and during recovery there was an additional depolarization of about 5 mV the mean E_r at 112 min being −80.0 mV. In contrast, the effect on V_A was pronounced. From a control value of 655 V/s, V_A was reduced to 84 V/s after 15 min in TX 100 following recovery for 100 min in normal Ringer solution V_A was 480 V/s. The maximum value for a recovered fibre was obtained after 119 min, V_A being 560 V/s. It is thus uncertain if the effect is completely reversible or not. In any case the time required for complete recovery appears to be considerable.

To study in more detail the effect of TX 100 several concentrations from 10 to 320 μM were tested and the time course of the changes of E_r and the action potential (AP) were followed. In Fig. 4 the effect on E_r is shown before and during exposure at two concentrations (40 and 160 μM). As seen the E_r value decreases linearily with time of exposure. In 60 min E_r decreased to −83 mV at 40 μM and to −68 mV at 160 μM. Measurements (not illustrated in Fig. 4) at 80 μM and 320 μM showed that at the former concentration E_r was not reduced (from −88.0 to −88.0 mV in 60 min) and at the latter concentration there was a *linear* reduction in E_r from −89.0 mV to −68.6 mV. This suggests that increasing the concentration from 160 μM to 320 μM does not influence the rate of decrease in E_r. The reduction in E_r is thus not related to the concentration of TX 100 in a simple way.

The specific membrane resistance (R_m) and the membrane capacitance (C_m) were followed in one muscle exposed to 40 μM TX 100. A slight increase in R_m from 2 577 ± 622 Ωcm²

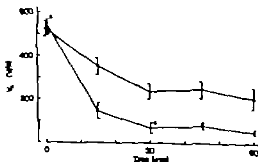


Resting membrane potential (E_r) ...curves) and peak height of action ... (upper curves) vs. time for ... treated with TX 100 for 60 min at ... ations of 40 µM (filled circles, solid ... 160 µM (open circles, broken line). ... ndles were exposed at 40 µM and ... ndles were exposed at 160 µM, each ... providing 20 to 40 surface fibres. ... ment within ±5 min from the times ... d was pooled and mean and stand... ation (S.D.) calculated. Number of ... ed are indicated by figures at each

to 3 790 ± 1 162 Ωcm² (= 5) was obtained. Values are mean ± standard error of the (S.E.). However statistically tested at a 5% level this difference is not significant. ... was estimated using the relation C_m R_m where was taken as the time for an ... ic potential change to reach 83% of full value (Hodgkin and Rushton 1946). C_m ... und to be 13.3 ± 4.5 µF/cm² (n 4) in normal Ringer solution and 10.3 ± 2.9 µF/cm ... after 60 min in TX 100 (mean ± S.E.). This difference is not statistically significant at ... level. The results suggest that TX-100 has little effect on passive permeability proper... ...nd that E_r linearly decreases with time t concentrations greater than 80 µM.

... effect of TX 100 on the dynamic membrane properties is illustrated in Fig. 4 (height ...) and in Fig. 5 (V_A). In contrast with the almost linear time course of decrease of E_r ...anges in height of the AP and V seem to follow a more exponential time course with ...dy state reached after about 30 min. It is most likely that the detergent affects E_r and ...tion potential through different mechanisms.

...ilar experiments following V for other concentrations from 10 to 320 µM were per



... Maximum rate of rise (V_A) vs. time ... concentrations of Triton X 100 40 µM ... line) and 160 µM (broken line). Points ... ean values and bars indicate ± S.D. of ... ean. Figures above or below the points ... e number of fibres.

Fig. 6

Fig. 7

Fig. 6. Relationship between maximum rate of rise and concentration of Triton X 100 $V_{A\,rel}$ is the tion between V_A after exposure to the actual concentration for 30 min and V_A before the applica Points indicate measured values. Curves are (1 − y) (see text) for 2 values of n, − 1 (first order reac and n − 2 (two TX 100 molecules reacting simultaneously with two sites). For additional information text.

Fig. 7 Hill plot. Log $y_e/(1-y_e)$ vs. log (TX 100) Correlation coefficient r = 0 98.

formed to obtain a dose–response relationship In Fig. 6 is plotted the reduction in m mum rate of rise (V_A) after 30 min ($V_{A\,rel}$) versus concentration of TX 100 From this gr the concentration for 50% reduction of V_A (ED_{50}) was found to be 36 µM which is equi lent to the value of the apparent dissociation constant ($K_{D\,app}$)

Steady state kinetics

To account for the effect of TX 100 on V_A it was assumed that TX 100 binds reversibly to some accep in the muscle membrane, probably receptor It was further assumed that one or more molecules of TX simultaneously react with this site (cf H bbard, Llinás and Quastel 1969) i e

$$TX-100+R \underset{k}{\overset{k}{\rightleftarrows}} TX-100\ R$$

where k and k_d are the association and dissociation rate constants respectively It is assumed that V_A the presence of TX 100 is proportional to the fraction of unoccupied sites, $1-y_e$, where y is the fracti of receptors occupied at equilibrium. From the law of mass action the occupied fraction of receptors equilibrium is given by

$$y = \frac{[TX-100]}{[TX-100] + K_D} \quad \text{(c.f H bbard et al. 1969 Rang 1971)}$$

From this the equilibrium dissociation consta t $K_D = k_d/k$ can be calculated according to

$$K_D = (1-y)/y \quad (TX\ 100)^n$$

Table I contains values for y and $(1-y_e)/y_e$, i the ratio of unoccupied sites t occupied sites. From th ratio and the concentration of TX 100 the equilibrium dissociation constant K_D for − 1 was calculat

Table I. Equilibrium inhibition of V_A by Triton X 100.

V_{rel} (%)	y_e	$\frac{1-y_e}{y_e}$	[TX 100] (M) 10^{-6}	^{10}log [TX 100]	K_D (= 1) (M) 10^{-6}	K_D (= 1.57) (M ^{n}) 10^{-6}
90	0.10	9.00	10	−5.00	90	12.7
74	0.26	[illegible]85	20	−4.70	57	11.9
45	0.55	0.82	40	−4.40	33	10.2
15	0.85	0.18	80	−4.10	14	6.7
13	0.87	0.15	160	−3.80	24	16.5
13	0.87	0.64	320	−3.50	12	13.0
				Mean ± S.D	38 ± 30	11.8 ± 3.3

according to equation (3). The mean ± S.E. was 38 ± 12 μM, but it is noted that the individual K_D values vary systematically, the highest values being associated with the lowest concentration of the detergent, thus indicating that n probably is not 1. To evaluate n a Hill plot (Hill 1909, Ariëns, Simonis and Van Rossum 1964) was used (Fig. 7). In this graph log $(1-y_e/y_e)$ versus log (TX 100) was plotted and n calculated, its value being 1.57 (correlation coefficient −0.98). This value deviates considerably from 1 i.e. the reaction is not of the type involving one ligand to one receptor site. K_D for n = 1.57 was also computed and incorporated in Table I, the mean ± S.E. being 11.8 ± 1.3 10^{-6} $M^{1.57}$ which is equivalent to 38 μM for 50% reduction in V_A. These findings indicate that if there exist two sites in the classical sense they are most likely interacting. However, other mechanisms are possible, which will be discussed later.

Comparison between TX-45, TX 100 and TN 101

In connection with the experiments with TX-100 some other detergents of the Triton series were studied for comparison with respect to their effect on E_r and V_A. The time course of the effect on E_r of TX-45, TX 100 and TN-101 is shown in Fig. 8. The standard deviation (S.D.) is less than ±8.0 mV, the mean value for the S.D. for all E_r being 4.8 mV. It appears from Fig. 8 that TX 100 has greater effect on E_r than TX-45. It is of interest to note that the slope of the regression line for TX-45 does not differ from zero, indicating that this detergent does not affect E_r.

In contrast to the effect on E_r all three detergents strongly reduce V_A (Fig. 9), this parameter being reduced to almost zero at 60 min for all detergents. It cannot be excluded that the effect differs somewhat with respect to the time course. The curves were drawn by eye and S.D. omitted as in Fig. 8. The mean absolute value of the S.D. for all points in this graph was 61 V/s with the maximum value for the S.D. being ±115 V/s. It is obvious that the detergents differ in their action on the frog muscle membrane, this difference being most marked with respect to their effect on E_r.

Discussion

The present study has provided evidence that nonionic detergents in the Triton series abolish the action potential in the frog muscle with small or no effects on the resting membrane potential. Passive electrical properties such as the specific membrane resistance (R_m) and the membrane capacitance seem to be very little altered by these detergents. The static kinetic properties of the TX 100-membrane system (depression of AP) were determined in

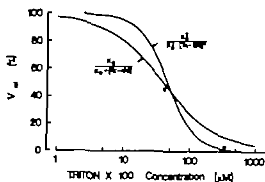


Fig. 6

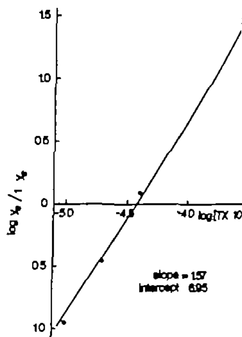


Fig. 7

Fig. 6. Relationship between maximum rate of rise and concentration of Triton X 100. $V_{A\,rel}$ is the rel tion between V_A after exposure to the actual concentration for 30 min and V_A before the applicatio Points indicate measured values. Curves are $(1-y_e)$ (see text) for 2 values of n $=1$ (first order reactio and n = 2 (two TX 100 molecules reacting simultaneously with two sites). For additional information, s text.

Fig. 7 Hill plot. Log $y_e/(1-y_e)$ = log (TX 100). Correlation coefficient r = 0.98.

formed to obtain a dose-response relationship In Fig. 6 is plotted the reduction in maxi mum rate of rise (V_A) after 30 min ($V_{A\,rel}$) versus concentration of TX 100 From this grapl the concentration for 50% reduction of V_A (ED_{50}) was found to be 36 μM which is equiva lent to the value of the apparent dissociation constant ($K_{D\,app}$).

Steady state kinetics

To account for the effect of TX 100 on V_A it was assumed that TX 100 binds reversibly to some acceptor in the muscle membrane, probably a receptor It was further assumed that one or more molecules of TX 100 simultaneously react with this site (*cf* Hubbard Llinás and Quastel 1969) *i.e*

$$n\,TX-100+R \underset{k}{\overset{k}{\rightleftarrows}} TX-100\ R \tag{1}$$

where k_1 and k_2 are the association and dissociation rate constants respectively It is assumed that V_A in the presence of TX 100 is proportional to the fraction of unoccupied sites, $1-y_e$, where y is the fraction of receptors occupied at equilibrium. From the law of mass action the occupied fraction of receptors at equilibrium is given by

$$y=\frac{[TX-100]^n}{[TX-100]^n+K_D} \quad \text{(c.f. Hubbard et al 1969 Rang 1971)} \tag{2}$$

From this the equilibrium dissociation constant $K_D = k_2/k$ can be calculated according to



$$K_D=(1-y_e)/y\ \ (TX\ 100)^n \tag{3}$$

Table I contains values for y and $(1-y_e)/y_e$, *i.e.* the ratio of unoccupied sites to occupied sites. From this ratio and the concentration of TX 100 the equilibrium *dissociation constant* K_D *for* $=1$ was calculated

... associated to the membrane may also be the target for detergent interactions. It is well known that detergents bind to both water soluble proteins and to more hydrophobic proteins, both in a ligand–receptor fashion according to the law of mass action or cooperatively with many detergent molecules interacting simultaneously (Helenius and Simons 1975, Tanford and Reynolds 1976). This association will often be followed by denaturation of the protein although this does not seem to be the case using "mild" detergents such as Triton X 100. Nevertheless, many membrane-bound proteins loose their biological activity without being denaturated which may either be due to a ligand–receptor interaction or a perturbation of the lipids in the vicinity of the protein by hydrophobic interactions between detergent and protein. The microenvironment of the proteins will thus change which will have consequences for their function (Tanford and Reynolds 1976).

As mentioned in the Results changes in E_r are not related to the concentration of Triton X 100 in a simple fashion. For concentrations up to 80 μM where the maximum rate of rise (V_A) is reduced to about 15 % of control (Fig. 6) a very small reduction in E_r is observed, while at 160 μM a linear depolarization with time from −88.6 mV to −67.5 mV in 60 min is found. Together with the fact that R_m and C_m at 40 μM TX 100 is approximately unchanged it is obvious that the passive permeability properties of the muscle membrane are little affected up to 80 μM of TX 100. If it is assumed that the lipid bilayer is mainly responsible for these passive properties (*cf.* Singer and Nicolson 1972) it can be concluded that the association of TX 100 to the membrane, does not impart any drastic changes in the physical properties of the lipid core, the resting permeabilities for different ions probably being normal. However, it is reported that Na-K-ATPase looses its biological activity after treatment with TX 100 (Tanaka and Abood 1964). A reduction of the pumping activity in the muscle membrane at high concentrations could well account for the observed slow linear depolarization seen in Fig. 8 (Draper, Friebel and Karzel 1963, Locke and Solomon 1967). The effect of TX 100 on the action potential is most likely to be attributed to a reduction of the initial sodium current as suggested by the measurements of V_A. The apparent dissociation constant K_{app} found in Fig. 6 is 36 μM and the corresponding value for K_D calculated from eq. (3) with 1.6 is 38 μM. The concentrations are in the same range as local anesthetics, *e.g.* lidocain reduces the sodium current in myelinated nerve fibres to 50 % at 100 μM (Hille 1966) and it is demonstrated that procain reduces the V of chicken heart muscle fibres to 50 % at concentration of about 100 μM (Josephson *et al.* 1976). The binding characteristics of the detergent as revealed from the dose response curve of V_A make it unlikely that the binding reflects an unspecific (*i.e.* lyothropic) interaction with the membrane lipids, since this would imply effects on the passive permeability which are demonstrated to be very small. A more plausible explanation is that the dose response curve reflects some interaction with proteins in the membrane eventually involved in the generation of the action potential. Such interaction is probably not of the cooperative type, referred to above, since it would give a much steeper dose response curve (Tanford and Reynolds 1976). The interaction can be better explained if it is assumed that the protein has some sites for the detergent, which may not necessarily be stereospecific but perhaps of the "group-specific" type (Monod, Wyman and Changeux 1965) with lower affinity. In view of the fact that many proteins integrated in the membrane have hydrophobic regions which may establish nonpolar inter-

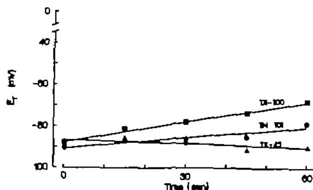


Fig. 8 Resting membrane potentı as time after application of three detergents t concentration of 16 Bars indicatı $\pm$ S.D are omitted for (see text). Straight lines are fitted points by a least square regression a The measurements were made from 2 (TX-45 and TX 100) and 3 muscles (T

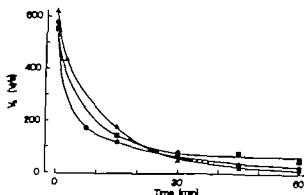


Fig. 9 Maximum rate of rise (V_A) after pplication of TX-45 (triangles), T (squares) and TN-101 (circles) at a conc tion of 160 μM Bars indicating S D been omitted for clarity (see text). Curv drawn by eye Same number of muscles Fig. 8.

two ways. It was not possible to describe the process as either a first or second order reac and n was estimated to 1 6

The action potential in the frog skeletal muscle fibre is the result of a fast inward soc current followed by a slowly developing outward potassium current (Adrian, Chandler Hodgkin 1970) Under physiological conditions V_A is a good estimate of the inward current (Hodgkin and Katz 1949 Schwartz, Ulbricht and Wagner 1973) Theoretically changes of V_A observed in the present study may be equivalent to one of following cha of ionic currents i) a decrease in sodium current, ii) an increase in potassium current, i summation of both The small alterations in potassium current on the frog nerve causec TX 100 (Brismar and Rydqvist, to be published) suggest that changes in V_A repre mainly the changes in sodium current.

As a background for discussing the present findings a brief summary of possible in actions between amphiphilic molecules and biological membranes will be given. Amp philes such as detergents are known to interact both with lipids and proteins although different ways. The interaction between detergents and phospholipids, which is the m lipid constituent of biological membranes, may result in lyothropic mesomorphism different liquid crystalline phases with diverse physical properties may occur mainly depe ing on the ratio of the components involved (Small 1968 Ekwall 1975) The liquid crys neat phase is the lamellar phase known to be present in the lipid bilayer in biological me branes (Small Bourges and Dervichian 1966, Small 1968) The detergents may alter t naturally occurring lipid state, e.g. from a fluid liquid crystalline state to a less fluid one even to a solid gel phase thus changing physical and consequently physiological properti of the lipid bilayer

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actions with the detergent it is conceivable that the detergent may substitute for nat occurring membrane lipids. This would require more "sites" for TX 100 than is pre from the steady state kinetics. In terms of classical occupancy theory the observed e compatible with the assumption of two sites which are mutually interacting. An othe sibility that could account for the shape of the dose–response curve is the existence intermediate step between the primary interaction and the actual effect. Rang (197 proposed that the shape of the dose–response curves could be accounted for if several a group of *m* sites must be occupied to exert the effect. A more likely explanation is th effect seen, is a result of the interaction between the detergent and two or more disti sites which mutually influence their affinity for the detergent, *i.e.* allosteric theory (M *et al* 1965) However the present findings cannot distinguish between these alternati

Finally a comparison between the effects of the three detergents used (TX-45 T and TN 101) reveals little difference in their action on the frog muscle membrane. Th clear difference is that TX-45 which is a more hydrophobic detergent (HLB number does not seem to depolarize the cell membrane. This may be accounted for by a dif interaction with the membrane lipid phase due to its hydrophobicity An equally factory explanation may be that this detergent does not interfere with the sodium- (Na–K-pump)

In summary the detergents TX 100, TX-45 and TN 101 seem capable of abolishi action potential in muscle by reducing the early sodium current without any drastic on passive electrical properties. It is suggested that this effect is mediated via a perturb of one or more proteins in the membrane either in a drug–receptor mechanism inv two (or more) sites or a more unspecific interaction to hydrophobic loci on the prote

I am greatly indebted to Professor D Ottoson, Dr E. Borg, Dr Å. Flock and Eng. B. Johansson fo able criticism and suggestions during the preparation of the manuscript.

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Methods

[illegible] was operated under ketamine-pentobarbital anaesthesia, with strict aseptic precautions. The right [illegible] artery and the right external jugular vein were cannulated with silicone rubber catheters, their tips [illegible] the aortic arch and the right atrium, respectively. The other catheter ends [illegible] were drawn out through [illegible] over the back of the skull. The latter was opened over the occipital and parietal lobes. The major [illegible] of the cerebrum [illegible] was removed, except for the frontal lobes and the diencephalon after which the brain- [illegible] was cut between the colliculi with [illegible] blunt spatula.

[illegible] subsequent days the animals received electrolytes, glucose solution, amino acids and lipid suspen- [illegible] Haematocrit, serum potassium and sodium were followed daily and, if necessary corrected. Over- [illegible] prevented by [illegible] fan, regulated by [illegible] rectal thermistor keeping body temperature at about 35°C [illegible] experimental observations were, however performed at [illegible] body temperature of 38°C.

[illegible] 1–7 days, the animals [illegible] were tracheostomized and [illegible] cannula with [illegible] thermocouple was put in the [illegible] permitting [illegible] recording of the ventilation rate. Arterial pressure was measured by connecting the [illegible] cannula to [illegible] P23 AC transducer and heart rate was obtained by an ordinate writer triggered by the [illegible] pulses. All recordings were made on [illegible] Grass polygraph. In 2 cats, the vagi were dissected free in the [illegible] so that they could be cut in the course of the experiment.

[illegible] simulated dive was to be performed, the animal was put with the ventral side down on [illegible] board, [illegible] head over the edge. A jar with water was lifted up from below until the nose and eyes were sub- [illegible] while the animal could ventilate freely through the tracheal cannula.

[illegible] cats, 1 ml of [illegible] water (15–20°C) [illegible] was instead injected into [illegible] nostril through [illegible] thin polyethylene [illegible] The animal was then lying on its side and was not tracheostomized.

[illegible] addition, 3 cats were acutely decerebrated under ether anaesthesia, to be used in [illegible] head submersion [illegible] the same day.

[illegible] all expts [illegible] the brain stems were fixed in formalin and paraffin-embedded. Sections, 20 μm thick, [illegible] cut and stained [illegible] with the Luxol fast blue-neutral red method. The sections [illegible] were inspected under [illegible] microscope to confirm that the neuraxis had been completely cut at the intended level, between the colliculi.

Results

[illegible] chronically decerebrated cats were subjected to head submersion. Of these 9 responded [illegible] apnoea and bradycardia whereas 2 cats did not display this type of responses. The latter [illegible] were tested already on postoperative day 1 and 2, as compared to a mean of 5.4 ± 1.7 [illegible] days for the others. In 7 animals, the reflex response was entirely reproducible through- [illegible] and in the other 2 (postoperative day 7 and 3), it was observed in about half of the [illegible]

[illegible] arterial blood pressure was maintained or slightly increased during the "dives". Apnoea [illegible] bradycardia always occurred together (see Fig. 1). The latency of the response was [illegible] s which could vary even in the same animal but was usually below 15 s. Thus, the [illegible] of the face did not immediately elicit the reflex response. Instead, it seemed often [illegible] occur first after some water had reached the nasal cavity or possibly other parts of the [illegible] respiratory passages. Once the response had started, however it was sustained and [illegible] lasted during a submersion of up to 60 s. After interruption of the "dive" [illegible] apnoea and [illegible] bradycardia vanished within 10 s. The reflex adjustments were not elicited by postural [illegible] as the animals kept the same position throughout the tests. The responses occurred [illegible] independently of water temperature (10–30 C).

[illegible] contrast, 3 acutely decerebrated cats subjected to submersion of the head displayed [illegible] neither apnoea nor bradycardia.

[illegible] The reflex bradycardia was very prominent in the [illegible] chronically [illegible] decerebrated cats. In

Acta physiol scand. 1977 101 476–480

From the Department of Physiology University of Göteborg, Sweden

Apnoea and Bradycardia from Submersion in "Chronically" Decerebrated Cats

By

Jan Martner, Hans Wadenvik and Björn Lisander

Received 13 June 1977

Abstract

Martner, J H Wadenvik and B. Lisander. *Apnoea and bradycardia from submersion chronically" decerebrated cats* Acta physiol scand 1977 *101* 476–480

In chronically but ot acutely decerebrated cats, submersion of the head caused apnoea and mar bradycardia, associated with a maintained or slightly raised arterial pressure. Since these reflex adjustme though very reproducible, occurred with varying latency and could be induced lso by nasal injectio water they appeared to be, at least in part, elicited from the upper respiratory passages. Thus, a terrest mammal, reputed t shun y f rm of immersion, can exhibit adjustments during head submersion, sim t those habitually diving species This response pattern is basically organized at the lower brainst level.

Submersion in water causes apnoea, bradycardia and peripheral vasoconstriction, not o in air-breathing aquatic animals but also in terrestrial species such as dog and man In t duck this response pattern can be elicited by stimulation of receptors in the upper respirato passages (Blix, Rettedal and Stokkan 1976) The resulting adjustments appear to be the r result of a complex interaction between the respiratory and cardiovascular centres (Angell James and de Burgh Daly 1972 a, Blix 1975)

In the duck the basic elements of the diving response remain after decerebration (Hux 1913 Andersen 1963 Djojosugito Folkow and Yonce 1969) However it has been infer that cortical influences are prominent in mammals and may even dominate the even Thus, seals often display an anticipatory bradycardia before a dive and also increase th heart rate when approaching the surface (Jones *et al* 1973) Further if the animal decid to interrupt the ascent and continue the dive, the bradycardia may return (Murdaug Seabury and Mitchell 1961)

Therefore, it appeared of interest to study whether the isolated brain stem of mammals capable of mediating a well organized diving reflex and to what extent this is the case al in notorious non-divers. For such purposes "chronically" decerebrated cats were expose to simulated diving by means of head submersion

(1963) proposed that the corresponding reflex adjustments in ducks are elicited by receptors on the outside of the head. However more recent studies suggest that receptors in the respiratory passages, especially in the glottis region, are of particular importance (Bamford and Jones 1974, Blix, Rettedal and Stokkan 1976). In the duck such receptors are immediately wetted by water as it cannot completely close the beak (Blix, Rettedal and Stokkan 1976).

In conscious trained dogs, voluntary snout immersion causes apnoea and bradycardia (Elsner, Stone and Young 1974). Water flow through the nose gives the same effect in anaesthetized dogs (Angell James and de Burgh Daly 1977 b). It is likely that submersion may have activated similar receptors in the decerebrated cats. To elucidate this, water was injected into one nostril which caused immediate apnoea and bradycardia in some expts.

In this context it should be noted that several other stimuli, besides water, when applied to the nasal cavity can elicit a similar response pattern. Thus cigarette smoke causes apnoea and bradycardia in the rabbit and this is still the case after decerebration (White and McRitchie 1973).

The apnoea and bradycardia always occurred together in the present expts. The decrease in heart rate took place immediately after the onset of apnoea even if it tended to become intensified during prolonged submersion. Several links between the apnoea and bradycardia have been envisaged (*cf* Angell James and de Burgh Daly 1977 a). Thus, the water sensitive receptors may elicit a *primary* reflex excitation of the vagal cardioinhibitory fibres. But the bradycardia can also be induced *secondarily* either by direct pathways between the respiratory and cardiovascular neuron pools in the brain stem or *via* reflexes from pulmonary and thoracic receptors. The bradycardia may be enhanced by an increased activity in unmyelinated vagal afferents from cardiac receptors, activated by the peripheral vasoconstriction (Blix, Wennergren and Folkow 1976). During prolonged submersion, arterial chemoreceptors are brought into play by the gradual fall in blood oxygen content, still more accentuating the bradycardia as long as apnoea prevails. As several of these reflexes have their afferent pathways in the vagi, vagotomy would have the double effect of abolishing both an efferent and several afferent links in this integrated reflex response. It is therefore not surprising that vagotomy greatly changed the reflex response to head submersion, with a reversal of the bradycardia to a weak tachycardia.

Thus, submersion of the head of decerebrated cats caused apnoea and bradycardia, indicating that these reflex effects do not require higher levels of the nervous system. This certainly does not deny that this basic submersion response can be considerably modified by higher nervous levels in intact animals. Indeed, there is ample evidence that this is the case, especially in diving species (Murdaugh, Seabury and Mitchell 1961, Jones *et al.* 1973).

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Thanks are due to Mrs Kerstin Bongsell for skilful technical assistance.

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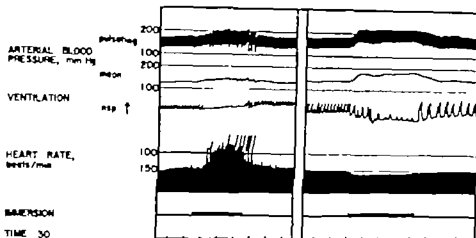


Fig. 1 Cat, 2.2 kg, postoperative day 3 I the first panel, submersion of the head causes apnoea a bradycardia with some latency After cutting of the vagi in the neck (second panel), the submersion apno is interrupted by inspiratory gasps and the bradycardia is abolished.

the majority of expts. heart rate fell from 150–200 beats/min to below 100. During th prolonged submersions, there was an accentuation of the bradycardia after 15–20 s.

Bilateral vagotomy abolished the bradycardia during the apnoea and there was instead moderate tachycardia and an intensified rise in arterial pressure (2 cats) In the experimen illustrated in Fig. 1 the apnoea was entirely reproducible and sustained during 8 trials befor vagotomy After cutting of the vagi, however there were inspiratory gasps during submer sion.

4 cats were exposed to water instillation in the nasal cavity In 2 cats (postoperative da 1 and 2), this immediately elicited apnoea and bradycardia lasting for 10–25 s, whereas i the others, no responses were elicited (postoperative day 4) The 2 responsive animals wer some days later tracheostomized and subjected to head submersion which regularly elicite apnoea and bradycardia.

Discussion

In the majority of the "chronically" decerebrated cats, submersion of the head caused respiratory arrest and marked bradycardia. Similar findings have previously been made in acutely decerebrated ducks (Huxley 1913 Andersen 1963 Djojosugito Folkow and Yonce 1969) These adjustments, very much reminding of those occurring during spontaneous diving in several species, can thus be mediated by lower levels of the central nervous system, not only in the duck but also in a mammal that is certainly far from being a habitual diver These responses were, however not observed in acutely decerebrated cats, but only in animals allowed to recover for some days after the truncation Apparently decerebration entails a reversible trauma to the central nervous system, besides the obvious irreversible injuries (Monakow 1914 p 26).

It is not clear exactly which type of receptors that were responsible for the reflex response to submersion in the present expts. It did not always occur immediately after submersion but appeared instead to be related to water reaching the upper respiratory passages. Andersen

proposed that the corresponding reflex adjustments in ducks are elicited by receptors outside of the head. However more recent studies suggest that receptors in the re ry passages, especially in the glottis region, are of particular importance (Bamford ea 1974, Blix, Rettedal and Stokkan 1976). In the duck such receptors are immediately l by water as it cannot completely close the beak (Blix, Rettedal and Stokkan 1976). onscious trained dogs, voluntary snout immersion causes apnoea and bradycardia s, Stone and Young 1974). Water flow through the nose gives the same effect in tized dogs (Angell James and de Burgh Daly 1972 b). It is likely that submersion ve activated similar receptors in the decerebrated cats. To elucidate this, water was d into one nostril which caused immediate apnoea and bradycardia in some expts. is context it should be noted that several other stimuli, besides water when applied asal cavity can elicit a similar response pattern. Thus cigarette smoke causes apnoea adycardia in the rabbit and this is still the case after decerebration (White and McRit 973).

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Acta physiol. scand. 1977 101 481–488

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Role of the Adrenergic Nervous System in Development of Training-Induced Bradycardia

By

K. Sigvardsson, E. Svanfeldt and Å. Kilbom

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Abstract

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Sprague-Dawley rats, normal and chemically sympathectomized with 6-hydroxy-dopamine, were trained by treadmill running. The normal rats, unlike the sympathectomized animals, showed reduction of the exercise heart rate after the training period. Compared to a sedentary control group the sympathectomized rats showed no difference in intrinsic heart rate after pithing and denervation and no increase in heart weight. The increase of the heart weight/body weight ratio after training was smaller in the sympathectomized group than in the normal one. The results show that a functioning adrenergic nervous system is necessary for an optimal adaptation to physical training. Administration of noradrenaline to pithed trained and untrained rats showed that beta-adrenergic receptor sensitivity was not altered by physical training. The intrinsic heart rate of normal trained rats was lower than that of normal control rats.

Key words: Physical training, intrinsic heart rate, 6-hydroxy-dopamine, receptor sensitivity, pithing

The physiological adaptation to physical training includes a variety of adjustments in the mechanisms of uptake, transport and utilization of oxygen.

The main changes in cardiac performance after training are a decreased heart rate and an increased stroke volume at rest and during exercise (*cf.* Åstrand and Rodahl 1970). However the mechanisms producing these adjustments are still poorly understood. It has been shown that the activity of the autonomic nervous system is altered during training and several authors claim that these changes are involved in the development of training bradycardia.

Thus the training bradycardia has been attributed to an increased cardiac concentration of acetylcholine (Tipton and Taylor 1965) or to a decreased cardiac catecholamine concentration (de Schryver *et al.* 1967, 1969). Östman and Sjöstrand (1971) however using a far more strenuous training program, found no difference in cardiac noradrenaline concentration between trained and untrained rats.

In training expts. where autonomic blockade was performed the sympathetic activity seemed to have decreased after training (Ekblom *et al.* 1973 b, Frick *et al.* 1967, Herrlich *et al.* 1960).

The importance of the autonomic nervous system for the development of training brady cardia can also be analyzed by eliminating the *different constituents of the autonomic* nervous system in animals. If these animals are trained, and compared to a group of trained, *nontreated controls*, the character of any differences in the adaptation to physical training can be used to estimate the significance of the excluded parameter

In this study the principal aim was to elucidate the role of the adrenergic system in the adaptation to physical training, mainly the post training bradycardia. The method chosen was to study the heart rate response to training both in normal rats and in rats subjected to *sympathectomy The sympathectomy was induced chemically with* 6-hydroxy-dopamine (6-OH DA), which causes a long lasting degeneration of the adrenergic nerves (de Champlain and Nadeau 1971 Malmfors and Thoenen 1971 de Champlain and van Ameringen 1972, Gauthier *et al* 1972).

The heart rate effect of physical training may not be entirely attributable to changes in autonomic nerve activity The "*intrinsic heart rate*"*—whether it can be explained by hyper* trophy increased contractility or in other terms—may also be influenced by physical training. In this study pithing (Shipley and Tilden 1947) was performed in order to eliminate the cerebral and spinal influence on the circulatory system. It was used in combination with 6-OH DA treatment and bilateral vagotomy in both the normal and the previously treated rats in order to obtain a preparation completely depleted of nervous influence on heart rate, the heart still remaining in the circulatory system of the body

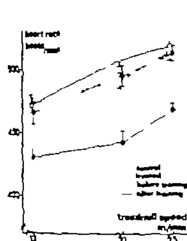
Some authors have suggested that the sensitivity of the adrenergic beta-receptors decreases during physical training. Crews and Aldingen (1967) when measuring isometric systolic tension in anesthetized rats, found that hearts of trained rats responded less to a given dose of exogenous noradrenaline than did those of untrained rats. Our finding (Ekblom *et al.* 1973a) of a less pronounced acute effect of 6-OH DA in trained rats also seemed to support this *theory Pavlik et al* (1976) *however* have found experimental support for a lower beta-activity but no marked changes in receptor sensitivity after physical training. In the present investigation, noradrenaline in *increasing concentration* was administered *to rats previously* subjected to pithing, 6-OH DA treatment and vagotomy in order to determine the heart rate reaction to noradrenaline when no nervous mechanisms could interfere.

Methods

25 *female rats*, of which 12 had been treated with 6-OH DA (50 mg/kg i p) as newborn (Nadeau *et al* 1971) were trained by treadmill running at a speed of 25 m/min for 1 h a day 5 days a eek, for 12 eek Training started when the animals were about 3 mo ths old Another 25 female rats, from the same litters as the exercised animals, served as controls. I this group, too, 12 of the rats had been sympathectomized a few days after birth

Two months after birth, three small *silver electrodes were implanted subcutaneously on the back of each* rat. These electrodes could easily be connected t cables for ECG-record ngs du g exercise (Ekblom *et al.* 1973a).

Before and after the training period all rats were exercise tested nd during three days prio to the tests all rats were exercised for about / h a day in order to accustom them t treadmill running. E ercise heart rates were measured with the rats running on the treadmill at three different speeds, 12. *I and 33.5 m/mi* respectively at an inclination of about 3



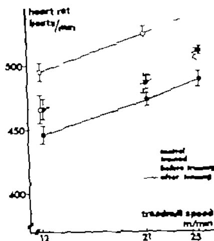


Fig 1 Fig 2

Fig. 1 Normal rats. Heart rates during exercise at 3 different work loads before and after the training period. Mean values and S.E. are given. Open circles: control rats, filled circles: trained rats.

Fig. 2 Sympathectomized rats. Heart rates during exercise at 3 different work loads before and after the training period. Mean values and S.E. are given. Open circles: control rats, filled circles: trained rats.

The rats were exercised for 10 min at the lowest speed and subsequently for 5 min each at 21 m/min and 25 m/min. Heart rate was recorded in 10 s periods 3 times during the last 3 min of each work load, and the average was used. During the training and testing period, 6-OH-DA treatment, 50 mg/kg was repeated every third week (de Champlain 1971). After the final work tests, all rats, both normal and sympathectomized, were treated with 6-OH-DA, 50 mg/kg. On the following day they were anaesthetized with ether and then subjected to pithing and bilateral cutting of the vagus nerves in the cervical region. The animals were artificially ventilated and the external jugular vein was cannulated. A polyethylene catheter was also inserted into the carotid artery and connected to a Statham P 23 DC transducer for blood pressure recording on a Grass Polygraph. Through the venous catheter increasing doses of noradrenaline (NA, norepinephrine) were injected and the heart rate and blood pressure responses to these injections were recorded. The NA infusion lasted about 30 min, after which the hearts and adrenals were removed and weighed.

Statistical analysis

Whenever possible, i.e. in comparisons of exercise heart rates before and after training, the *t*-test for paired observations was used. In other cases, e.g. in comparisons of heart weights between groups, the significance of differences between means for different groups was calculated using Student's *t*-test for independent samples. Level of significance, unless otherwise stated, $p < 0.001$.

Results

In the normal rats, the exercise heart rates of the training and the sedentary groups were identical before the training period. After training, the heart rates of the trained rats were significantly lower at all work loads than those of the controls and also compared to the pre-training values (Fig. 1).

In the sympathectomized group the trained rats had lower exercise heart rates than the controls after the training period, the difference being 40–50 beats/min (same as in the normal

TABLE I

Group	Body weight, g	Heart weight	Heart weight/ body weight, %	Adrenal weight, g	Adrenal weig body eight,
Normal control (n = 12)	285 ± 5	0.935 ± 0.014	3.293 ± 0.069	0.031 ± 0.001	0.110 ± 0.003
Normal trained (n = 12)	283 ± 4	1.079 ± 0.026	3.811 ± 0.062	0.034 ± 0.001	0.119 ± 0.002
6-OH DA control (n = 12)	286 ± 5	0.921 ± 0.024	3.212 ± 0.045	0.032 ± 0.001	0.111 ± 0.002
6-OH DA trained (= 10)	266 ± 6	0.942 ± 0.010	3.556 ± 0.059	0.035 ± 0.001	0.131 ± 0.003

Body weight, heart weight and adrenal weight after the training period. Mean values and S.E. are gi
The symbols indicate the significance of d fferences between the trained groups and their respec control group
p. < 0.05 p < 0.01 p < 0.001

group) However the difference in this case was due not so much to a lowered heart rate the trained rats as to a rise in the controls (Fig. 2).

At the beginning of the training period no differences in body weight existed between t groups (mean 242 g) During the training period all groups increased their body weight b the trained 6-OH DA treated rats to a smaller extent than the other groups (Table I) On the normal trained rats had a significantly greater heart weight than their controls. The hea weight/body weight ratio was significantly higher in both normal and sympathectomiz trained rats compared to their respective control groups. The difference was however small between the sympathectomized groups and reflected the difference in body weight. Th difference in the heart weight/body weight ratio between trained normal rats and train sympathectomized rats was significant (p < 0.005), see Table I

The weight of the adrenal glands of the trained, denervated group was significantly great than that of the denervated control group (p 0.01), while there was no significant differen between the normal groups.

Concerning the adrenal weight/body weight ratio the difference between denervate trained and untrained animals was even more pronounced (p 0.001) and there was a sligh difference between the normal groups too (p 0.05)

In the normal group the preinjection heart rates were significantly lower in the traine rats (Fig. 3) The magnitude of the HR increase in response to noradrenaline injection wa the same in the trained and in the sedentary group At some doses the HR increase was eve slightly higher in the trained group (Fig. 3)

In the rats sympathectomized as newborn, the preinjection heart rates did not differ significantly between the trained and untrained groups. The HR response to exogenous nor adrenaline was somewhat lower than that of the non treated rats.

The blood pressure levels after pithing were not significantly different in normal an sympathectomized rats. The elevation of systolic and diastolic blood pressure after nor

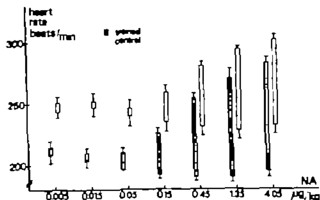


Fig. 3 Effect of NA infusion on heart rate of normal rats. The bottom of each column represents the preinjection heart rate − S.E. and the top represents the maximal heart rate response + S.E. The animals are pretreated with 6-OH-DA, vagotomized and pithed before these measurements.

adrenaline administration was the same in all four groups previously sympathectomized and normal, trained and untrained (Fig. 4).

Discussion

In previous training expts. with rats, only heart rates at rest have been measured (Tipton and Taylor 1965, Pavlik *et al.* 1976). Other investigators have performed graded treadmill tests in rats, but not in connection with physical training (Barnard *et al.* 1974, Denimal *et al.* 1970, Bonner *et al.* 1973).

When discussing the exercise heart rates before and after training in the different groups, the changes in body weight during the training period should be considered. Due to the increase in body weight of all groups during the training period, the post-training test at a 3° inclination must have been somewhat more strenuous. The effect seems however to have been modest, since the rats in the normal control-group had increased their exercise heart rate only slightly (5–10 beats/min, p 0.05) after the training period. Likewise, it may be argued

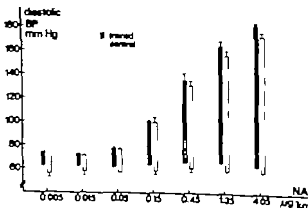


Fig. 4 Effect of NA infusion on the diastolic blood pressure of normal rats. The bottom of each column represents the pre-injection BP − S.E. and the top of each column the maximal pressure response + S.E. The animals were treated with 6-OH-DA, vagotomized and pithed before these measurements.

TABLE I

Group	Body weight, g	Hea t
Normal control (n = 12)	285 ± 5	0 935 _
Normal trained (n = 12)	283 ± 4	1 079 ± 0
6-OH DA control (n = 12)	86 ± 5	0.921 ± 0 0
6-OH DA trained (n = 10)	66 ± 6	0 942 ± 0 010

Body weight, heart weight and drenal we ght f
The symbols indicate the significance of diff
control group.
p. < 0 05 p < 0.01 p < 0 001

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the normal trained rats had a significantly greater l
weight/body weight ratio was significantly higher i
trained rats compared to their respective control grou
between the sympathectomized groups and reflecte
difference in the heart weight/body weight ratio betw
sympathectomized rats was significant (p 0 005) see I

The weight of the adrenal glands of the trained, deners
than that of the denervated control group (p 0 01) whil
between the normal groups.

Concerning the adrenal weight/body weight ratio th
trained and untrained animals was even more pronounced (
difference between the normal groups too (p 0 05)

In the normal group the preinjection heart rates were si
rats (Fig. 3) The magnitude of the HR increase in response t
the same in the trained and in the sedentary group At some d
slightly higher in the trained group (Fig. 3).

In the rats sympathectomized as newborn, the preinjectio
significantly between the trained and untrained groups. The HR r
adrenaline was somewhat lower than that of the non-treated rat

The blood pressure levels after pithing were not significantl
sympathectomized rats. The elevation of systolic and diastolic l

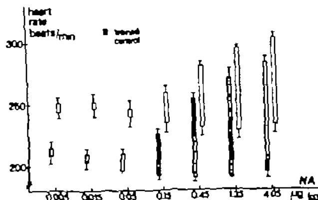


Fig. 3. Effect of NA infusion on heart rate of normal rats. The bottom of each column represents the pre-injection heart rate ± S.E. and the top represents the maximal heart rate response ± S.E. The animals were pretreated with 6-OH DA, vagotomized and pithed before these measurements.

atropine administration was the same in all four groups: previously sympathectomized and normal, trained and untrained (Fig. 4).

Discussion

In previous training expts. with rats, only heart rates at rest have been measured (Tipton and Taylor 1965, Pavlik *et al.* 1976). Other investigators have performed graded treadmill tests in rats, but not in connection with physical training (Barnard *et al.* 1974, Denimal *et al.* 1970, Wyatt *et al.* 1973).

When discussing the exercise heart rates before and after training in the different groups, the changes in body weight during the training period should be considered. Due to the increase in body weight of all groups during the training period, the post-training test at a 3 inclination must have been somewhat more strenuous. The effect seems however to have been modest, since the rats in the normal control-group had increased their exercise heart rate only slightly (5–10 beats/min, $p < 0.05$) after the training period. Likewise, it may be argued

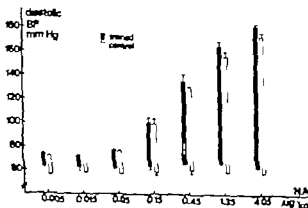


Fig. 4. Effect of NA infusion on the diastolic blood pressure of normal rats. The bottom of each column represents the pre-injection BP ± S.E. and the top of each column the maximal pressure response ± S.E. The animals were treated with 6-OH DA, vagotomized and pithed before these measurements.

that since the exercise heart rates of the trained denervated rats were obtained at a body weight 10 percent lower than in the other three groups, the training effect in this group was somewhat exaggerated It was actually only in the normal group that the exercise heart rate was significantly reduced after training. The lack of a similar effect in the sympathectomized group must be related to the 6-OH DA treatment The sympathectomy did not impair the ability to increase heart rate, so the lack of training effect in these rats was not associated with an inability to reach high heart rates during training. On the contrary treated rats have been shown to have higher heart rates during exercise than normal rats (Ekblom *et al* 1973a). Also at rest we have found a higher heart rate in 6-OH DA treated rats than in normal ones (unpublished results) The higher heart rate of denervated rats at rest and during exercise is not fully understood but some possible factors could be mentioned

1 6-OH DA treatment causes an adrenergic denervation of the blood vessels, resulting in an impairment of the circulatory control and a blood pressure reduction (de Champlain and van Ameringen 1972, Gauthier *et al* 1972), which is partly compensated for by an increased adrenal catecholamine production

A contribution to the high heart rate could come from the "supersensitivity" to catecholamines that has been shown to occur after sympathectomy (Nadeau *et al* 1971)

2. Because of the impaired circulatory control the sympathectomized rats would require a greater cardiac output than normal rats to ensure the working muscles an adequate blood supply

Thus, the increased heart rate of the denervated control group after the training period could reflect a progressive impairment of the circulatory function. In the denervated training group this impairment seems to have been counteracted by the training, although the adaptation to training was less efficient than in the normal trained group

In the pithed and denervated specimens there was no neural influence on heart rate and we have therefore used the term "intrinsic" for the heart rates obtained from these. In the present investigation the intrinsic heart rate was significantly reduced in the trained normal group whereas no difference in intrinsic heart rate was observed between trained and untrained sympathectomized rats

Differences were also obtained in the development of heart weight and body weight between the trained sympathectomized and the trained normal group Only the normal rats showed an absolute increase in heart weight after training compared to the respective sedentary group The sympathectomized trained rats had a lower mean body weight than the other groups. Therefore, their heart weight–body weight ratio was greater than in the denervated control group although smaller than in the normal trained group.

Similar results have been reported by Tipton (1965) from measurements of heart weight, body weight and heart weight/body weight ratio in training expts. with immunosympathectomized rats

All these parameters—heart weight and heart weight/body weight ratio exercise heart rate and intrinsic heart rate—show that the abolition of the adrenergic nervous system by 6-OH DA treatment at birth and during the training period alters the adaptation of the circulatory system to physical training.

One or several factors affecting heart rate after pithing and denervation must have caused

i difference between trained and untrained normal rats. Conceivable factors are intra-
ardial mechanisms, circulating adrenal catecholamines or other blood-borne agents, or
erations of the receptor sensitivity to catecholamines. The hypothesis of a decreased
renergic receptor sensitivity as a cause of the post-training bradycardia can be rejected,
ce the response to exogenous noradrenaline (increased heart rate and increased blood
essure in pithed rats) was the same in trained and untrained rats. The threshold dose, *i.e.*
e lowest dose of noradrenaline which gives a significant response, was also the same in
ined and untrained rats.

The alterations of the autonomic nerve function reported from investigations with auto-
omic blockade in trained animals and humans (see introduction) do not seem to be the
ure and fundamental explanation for the post-training bradycardia. This investigation has
own that trained rats still had their bradycardia after pithing, vagotomy and sympathec
my. This reduced intrinsic heart rate will have to be considered in the interpretation of
udies with autonomic blockade and it might explain the divergent opinions expressed by
me authors, investigating the influence of training on autonomic nerve activity. Further
ore, the development of this reduced "intrinsic" heart rate through training seems to re-
uire an intact adrenergic nervous system, since in sympathectomized trained rats there was
o such reduction.

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From the Department of Physiology, University of Goteborg, Sweden

...scular Reactivity to 5-Hydroxytryptamine (5-HT) in Hindquarter Vascular Beds, Aortic Strips and Portal Veins from Spontaneously Hypertensive and Normotensive Rats

By

Lars Åhlund, Yen Lundgren, Björn Sjöberg and Lilian Weiss

...ion studies on spontaneously hypertensive rats (SHR) illustrate the hemodynamic ...rtance of an altered precapillary vessel design in hypertension, extending earlier findings ...ential hypertension in man. Thus, dose-response relationships to noradrenaline (NA) ...ed an increased resistance even at maximal dilatation, a steeper "resistance curve" and ...creased maximal pressor response in SHR, but no difference in NA threshold com... ...d to normotensive control rats (NCR). Furthermore, aortic strips and portal veins from ...and NCR did not differ concerning NA sensitivity. The SHR and NCR resistance curves ...virtually identical with those of model resistance vessels, assumed to differ only in wall/ ...en ratios (*cf.* Folkow *et al.* 1973).

...oncerning another vasoactive agent, 5-hydroxytryptamine (5-HT), which in some cir... ...may contribute to ordinary vascular control, Haeusler and Finch (1972) observed only ...responses in hindquarter vascular beds and no real difference between SHR and NCR ...rning the mentioned resistance curve characteristics. According to Haeusler and Finch ...findings might challenge the concept that an altered resistance vessel design is of hemo... ...mic importance in hypertension. In perfused mesenteric arteries, on the other hand, they ...d an increased sensitivity and reactivity to 5-HT in SHR, while isolated renal arteries ...less responsive in SHR than in NCR. It should here be stressed that 5-HT exerts more ...plex effects than NA, constricting proximal resistance vessels (like the mesenteric artery) ...le dilating more distal ones, at least in some circuits (*cf.* Merrill *et al.* 1974). Further ...ructurally increased wall/lumen ratio may well be associated with independent changes ...smooth muscle responsiveness to vasoactive agents. The resistance curves are then, of ...se, influenced by at least two separate factors, but they can nevertheless often be ex... ...mentally distinguished (Folkow 1976). For such reasons it was considered of interest to ...her compare vascular smooth muscle responsiveness to 5-HT in SHR and NCR on two ...mmonly utilized proximal parts of the systemic circuit and on the hemodynamically all-... ...ortant microvessels.

...3 paired, constant-flow perfusions of isolated hindquarter vascular beds from adult (6–8 months) male ... and NCR (with mean arterial pressures of 150 ± 9 and 107 ± 2 mmHg, respectively) the pressor ...ses to increasing 5-HT concentrations were explored from maximal dilatation to maximal constric... (*cf.* Folkow *et al.* 1970). Once the responses to supramaximal 5-HT concentrations were reached ... as ...ed whether the resistance vessels could further constrict, by superimposing maximal doses of vasopressin

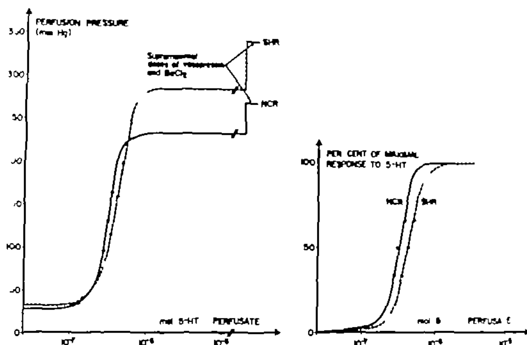


Fig. 1 Left part Mean dose-response curves for 5-HT from isolated hindquarter vascular beds of SHR and NCR, perfused at constant flow Right part Mea dose-response curves for 5-HT so recalculated that the respective maximal resistance increases in NCR and SHR are both set to 100 per cent.

and barium ions. — When strips of the aorta r isolated portal veins were studied, 9 pairs of these preparations from adult matched SHR and NCR were kept in the same organ bath and thus exposed to identical increments in 5-HT concentration (*cf* Hallbäck, Lundgren and Weiss 1971)

With respect to the 5-HT responsiveness of isolated aortic strips and portal veins from SHR and NCR both SHR preparations exhibited supersensitivity particularly the aortic strips. Thus, for SHR aortic smooth muscle ED_{50} was 1 4 ± 0 1 10 M compared with 1 4 + 0.3 10^{-6} M for NCR ($p < 0\ 01$) For portal vein the 5-HT sensitivity differed less between SHR and NCR, ED_{50} being 1 0 ± 0.2 10^{-6} M and 1 6 ± 0.2 10^{-6} M respectively (p 0 05)

Concerning the hindquarter resistance vessels the situation was quite different, as shown in Fig. 1 Evidently impressive resistance increases are induced by 5-HT both in the NCR and SHR hindquarter resistance vessels (left part) Further despite the higher distending pressure the SHR vessels clearly respond to 5-HT with a more extensive luminal narrowing than the NCR ones, as is the case also for NA vasopressin or barium ions, which latter two agents were given also here once the maximum 5-HT response was reached In the right part which better reveals a possible difference in 5-HT sensitivity between SHR and NCR there is a parallel shift to the right of the SHR resistance curve revealing a reduced 5-HT sensitivity in SHR ($p < 0\ 001$)

One difference from *e.g* NA responses is that the SHR and NCR resistance curves to 5-HT hardly differ in steepness, despite far higher maximal pressor responses in SHR (p 0 001). This may (Fig. 1 left part) be due to the lower 5-HT sensitivity in SHR (Fig. 1 right part), and/or the most distal hindquarter vessels may be poorly if at all constricted by 5-HT as observed in other species (Merrill *et al* 1974) In addition the 5-HT receptors may have a different distribution in the concentric muscle layers than the NA ones (Krishnamurty 1971) to some

vent affecting their relative contributions to luminal narrowing and hence to the curve shapes and its dependence on wall thickness. The NA influence is less complex since the adrenergic neuroeffector junctions, and probably most α-receptors have an adventitial location, from which neurogenic contractions are initiated, presumably engaging inner layers mainly by myogenic propagation (*cf* Ljung, Bevan and Su 1973).

In any case, it is clear *first* that 5-HT induces far stronger vasoconstrictor responses in the hindquarters than reported by Haeusler and Finch (1972), *second*, that the SHR resistance vessels regularly constrict more powerfully to high 5-HT concentrations than the NCR ones. However, independent of this resistance vessel "hyperreactivity" in SHR, the corresponding smooth muscles are, if anything, *less* sensitive to 5-HT than the NCR ones.

In conclusion, the situation concerning vascular smooth muscle sensitivity to 5-HT in NCR and SHR is quite complex. While aortic and portal vein smooth muscle (like mesenteric arteries) are more sensitive to 5-HT in SHR than in NCR, those of the true resistance vessels are less sensitive, though they nevertheless display the characteristic hyperreactivity that follows from an altered design. The present results thus illustrate that findings concerning smooth muscle responsiveness should not be generalized from one vascular preparation to the other; further that a structurally based "hyperreactivity" of SHR resistance vessels may well be separated from independent shifts in smooth muscle sensitivity. Therefore, the presence of various combinations of vascular design and effector responsiveness, with differences even between various cardiovascular regions, should always be borne in mind, particularly in analyses of hypertensive situations. In any case, the 5-HT supersensitivity in proximal vessels can hardly be of any direct relevance for the SHR hypertension, because the hemodynamically far more important distal vessels exhibit *reduced* sensitivity to 5-HT. The possibility remains that these apparently bizarre shifts in sensitivity indirectly reflect an alteration of some basic effector characteristic in SHR, which in other respects may be of considerable importance.

This investigation was supported by grants from the Swedish Medical Research Council (B77 14X-00016-13C), The Swedish National Association against Heart and Chest Diseases and the Medical Faculty University of Göteborg. AB Hässle generously covered part of the expenses for a technician.

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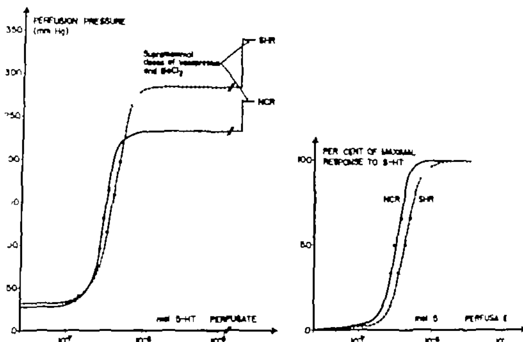


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Acta physiol. scand. 1977 101 492–494
From the Zoofysiological laboratory B Copenhagen, Denmark

Ca^{++} Sensitive Microelectrode: Intracellular Steady State Measurement in Nerve Cell

By

G R J CHRISTOFFERSEN and LEIF SIMONSEN

A Ca^{++} sensitive microelectrode of detection limit below intracellular activities was developed by Christoffersen and Johansen (1976) and shortly after by Brown *et al* (1976) Electrodes having tip-sizes down to 1 μm are now tested in neural somata of *Helix pomatia*. A steady state activity of 4 5 10 ⁷ M is concluded from the measurement.

The electrode was prepared as described by Christoffersen and Johansen (1976) except for two changes The PVC content was 10% instead of 40% and the external tip diameter was 1 μm instead of 20 μm

The electrode was calibrated against a calomel electrode in solutions of the same ionic strength as that of the Ringer solution (0 125) Solutions of pCa values 1.96 to 4.51 were NaCl–CaCl mixtures, single ion activity coefficient were calculated according to Butler (1968) Solutions of pCa values from 4.30 to 9 61 were made as Ca-EGTA buffers using 5 mM $CaCl_2$, 10 mM Na EGTA and adjusting pH with Tris-maleate buffer Concentration of Ca was calculated as the reciprocal $K_{MeL-total}$ in Caldwell (1970) and the Ca activity-coefficient was chosen equal to that of the $CaCl_2$–NaCl mixtures having the lowest Ca content

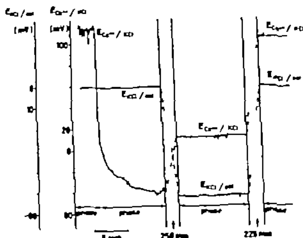
The potential difference ($E_{Ca/cal}$) *vs* pCa, was rectilinear in the pCa range 1.96 to 8.0 (*corresponding to the range* +71 *mV to* −98 *mV*) with a slope *s* of 28 0 mV/pCa (23 C).

The Ringer solution was that described by Kerkut and Thomas (1965) It has a calcium concentration of 7 mM $E_{Ca\ cal}$ *in the Ringer was 51 mV corresponding to a* Ca^{++} activity of 2.0 mM

3 electrodes were used for recording intracellular Ca-activity 1) A calomel electrode permanently placed in the bathing solution, 2) the Ca -sensitive electrode and 3) a glass microelectrode filled with 2.5 M KCl Potential difference recordings were made between 2 and 3 ($E_{Ca/KCl}$) and between 3 and 1 ($E_{KCl/cal}$)

The cell was the large medio-rostral cell in the right parietal ganglion.

The results from the experiment is shown in Fig. 1 In phase 1 both microelectrodes were placed in the Ringer solution, and $E_{KCl/cal}$ was adjusted to zero At phase 2 the Ca^{++} sensitive electrode impaled the cell At phase 3 the KCl-filled electrode also impaled the cell, subtracting the cell membrane potential − 53 mV from the $E_{Ca/KCl}$ level of phase 2, and at phase 4 both microelectrodes were withdrawn from the cell. The high noise level of the



recording of the potential difference, $E_{Ca^{++}/KCl}$, between Ca++ sensitive microelectrode and microelectrode, and the potential difference, $E_{KCl/Ref}$, between the KCl-filled microelectrode al electrode (placed in the Ringer' solution) the latter potential was counterbalanced to zero lectrodes were in the Ringer solution

recording in phase 1 resulted from attempts to penetrate the cell membrane. In $_{Ca^{++}/KCl}$ was constant within 6 mV for 8 h, and the $E_{Ca^{++}/KCl}$ difference between nd phase 4 was 10 mV

ion of intracellular Ca++ activity:

$$^{++}/KCl)_o \quad (E_{Ca^{++}/KCl})_i = \log \frac{a_{o,Ca} \quad K_{Ca,Mg}\, a_{o,Mg} \quad K_{Ca,} \quad a^{2}_{o,K} \quad K_{Ca,Na}\, a_{o,Na}}{a_{\;Ca} \quad K_{Ca,Mg}\, a_{i,Mg} \quad K_{Ca,} \quad a^{1} \quad + K_{Ca,Na}\, a^{1}_{i,Na}}$$

Walker (1971). $K_{Ca,}$ is the selectivity coefficient for the electroactive membrane i, $a_{o,}$ and $a_{i,}$ are extracellular and intracellular activities of these ions. π is the he calibration plot.

ity coefficients were obtained in the following way At pCa 7 6 (activity) $K_{Ca,Mg}$, d $K_{Ca,Na}$ were sought by adding 0.1 M (ionic strength) $MgCl_2$, KCl or NaCl to the uffer The resulting positive change of potential, being a sum of the response at ange sites in the electroactive membrane from the interfering ion species and an change in ionic strength, was corrected for the latter by adding a supposedly non-ng ion choline (0.1 M choline chloride) to an EGTA-buffer (pCa 7 6). Addi- the direct intracellular contribution from Mg to $(E_{Ca^{++}/KCl})_i$, (phase 3) was measured ng presumed intracellular activity of Mg (1 mM) to an EGTA-buffer of pCa) preliminarily approximated intracellular pCa obtained directly from $(E_{Ca/KCl})_o$ $_{Cl})_i$ using the calibration plot disregarding interference from foreign ions (pCa = 6.3). s of potential with addition of interfering ions are listed in Table I with calculated ity coefficients obtained from.

$$(E_{Ca} \;\; /KCl)_{\text{[illegible]}} = \log \frac{a_{Ca^{++}} \quad K_{Ca,} \; a_{i}^{\;n}}{a_{Ca^{++}}}$$

TABLE I Potential values from the Ca^{++} sensitive electrode for calculation of selectivity coefficients. Interfering ion species are added to EGTA buffers, changing ionic strength by 0 1 M. Resulting changes of potential are $\Delta(E_{Ca^{++}/cal})_{I\ varied}$. Effect of ionic strength is corrected for $(\Delta(E_{Ca^{++}/cal})_{I\ constant})$ by subtracting $\Delta(E_{Ca^{++}/cal})_{I\ varied}$ for 0.1 M choline from the same parameter for other ion species. All figures are mean of 3 expts. Mean variation between expts. was 32 % for a given parameter

Ion added to EGTA Buffer		$\Delta(E_{Ca^{++}/cal})_{I\ varied}$ (mV)	$\Delta(E_{Ca^{++}/cal})_{I\ constant}$ (mV)	p Ca	$K_{Ca,i}$
Choline	(0 100 M)	8		7 6	
N	(0 100 M)	17	9	7 6	33 10^{-4}
K	(0 100 M)	14	6	7 6	17 10^{-}
Mg^{++}	(0.033 M)	51	43	7.6	3 10^{-4}
Mg^{++}	(0.001 M)		2	6.3	5 10^{-4}

When values of $a_{o\ Ca}$ = 2 mM $a_{i\ Ca}$ = 5 10 M (pCa = 6.3) $a_{i\ N}$ = 4 mM (Thomas, 1972) $a_{i\ K}$ = 68 mM (measured with a K selective electrode) and extracellular values of other ions from the Ringer are inserted in eq 1 it reduces to the following significant terms.

$$(E_{Ca}\ /_{KCl})_o - (E_{Ca}\ /_{KCl})_i = \propto \log \frac{a_{o,Ca}}{a_{i\ Ca} + a_{i,MG} + a_{i,K}^2 K_{Ca,K}}$$

Setting $(E_{Ca}\ /_{KCl})_o - (E_{Ca}\ _{KCl})_i$ equal to the difference between the level of phase 3 and the mean of the level of phase 1 and phase 4 (Fig. 1) $a_{i\ Ca}$ emerges from eqn. 3 as 4.5 10^{-7} M in 3 expts. within the linear part of the calibration curve This value may be compared to 3 10 M in squid axons derived from aequorin fluorescence (Baker 1972) The behavior of the electrode in non-steady-state situations is being investigated

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The Intraerythrocytic Concentration of 2,3-Diphosphoglycerate in Type II A Hyperlipoproteinemia

By

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[It] has been proposed, that hypoxia is a major feature of arteriosclerosis (Hueper 1944), and that an increased erythrocyte oxygen affinity is a prominent feature in the production [of] arterial wall hypoxia (Brewer 1972). Rabbits on an atherogenic diet exposed to hypoxia [ha]ve higher cholesterol levels than control rabbits on an atherogenic diet (Kjeldsen *et al.* [19]68). Kjeldsen (1969) has reported a positive correlation between carboxyhemoglobin [le]vels and serum cholesterol levels in smokers. The increased risk of arteriosclerotic disease [in] cigarette smokers is well-established. The importance of 2,3-diphosphoglycerate for the [oxy]gen unloading properties of hemoglobin is well known (Chanutin and Curnish 1967). [Th]e overall profile of transmural oxygen tension in experimental atherosclerosis was marked[ly] different from normal (Heughan *et al.* 1973). The purpose of this study was to investigate [oxy]gen affinity of hemoglobin and 2,3-diphosphoglycerate concentration of red blood cells [in] patients of type II A hyperlipoproteinemia.

[...] patients had primary type II A hyperlipoproteinemia. The patients [w]ere kept on [a] proper diet for at [lea]st 3 months, and [t]hose [wi]th cholesterol values below 7.5 mmol/l [we]re excluded. All patients were clinically [sta]ble (3 females, 5 males, [a]ged ... 55 years). Two men [w]ere smokers. The venous blood samples were [tak]en after over-night fasting for determination of cholesterol, triglycerides, and phosphorus. Plasma low [dens]ity lipoprotein (LDL) isolation was carried out by [a] density gradient centrifugation method (Viikari [an]d Pelkonen 1974).

The oxygen affinity of the blood [w]as determined by equilibrating the blood sample [wi]th gas mixture [havi]ng constant and previously determined partial pressure of oxygen (26.3 mmHg) and carbon dioxide [(4]0 mmHg). The oxyhemoglobin saturation of the sample [wa]s then measured spectrophotometrically [acc]ording to Siggaard-Andersen *et al.* (1962). The oxygen affinity of the blood is expressed as standard [P_{50}] ([i.e.] the oxygen tension giving 50 [%] oxyhemoglobin saturation at pCO_2 40.0 mmHg and pH 7.40) and [wa]s calculated by means of Hill' equation (Rorth *et al.* 1972) and corrected to pH 7.40 according to [...] *et al.* (1963).

2,3-diphosphoglycerate (DPG) concentration of red blood cells was determined according to Rose and [Lie]bowitz (1970) and expressed as millimoles per liter of packed red blood cells. The values of intraerythro[cyt]ic 2,3-diphosphoglycerate concentration and standard P_{50} obtained from healthy [w]omen (aged 26–49), [mean] 31.9 years) and men (aged 22–55, mean 35.5 years) [w]ere used as controls.

[T]he main results of the study are shown in T[a]ble I. N[o] significant differences in the standard [P_{50} w]ere found. The DPG concentrations of the red blood cells of women were higher than [th]e mean values of the controls (p [<] 0.01), but the DPG concentrations of the red blood cells [o]f men [w]ere unchanged. Two women with the highest total and LDL-cholesterol concentra-

TABLE I. Serum cholesterol and 2,3-DPG in type II A hyperlipoproteinaemia

The patients had II A lipoprotein pattern on cellulose acetate electrophoresis. They were kept on hypocholesterolemic diet for at least 3 months before taking blood samples. 2,3-DPG of the female group differs significantly ($p < 0.01$) from the controls.

Initials	Sex	Age	Smoking	Serum cholesterol mmol/l	2,3-DPG mmol/l	Hemoglobin g/l	Phosphorus mmol/l	P_{50} mmHg
L. E.	♂	45	+(−)	11.9	4.9	138.3	1.1	25.2
L. M.	♂	28	−	11.6	4.6	148.7	0.9	25.2
H. K.	♂	32	−	9.1	4.4	152.6	0.5	24.9
L. T.	♂	4	+(−)	9.1	4.4	123.2	1.0	26.8
S. O.	♂	22	−	9.1	4.4	132.8	0.9	22.7
♂	30.2	22–45		10.2 ± 1.5	4.5 ± 0.2	139.1 ± 11.9	0.88 ± 0.23	25.0 ± 1.5
♂	35.5	22–55	Controls (n = 10)		4.7 ± 0.4			26.4 ± 1.4
T. L.	♀	49	−	13.4	5.8	101.7	1.2	25.8
S. M.	♀	55	−	12.6	6.6	110.2	1.3	25.3
K. L.	♀	41	−	8.7	4.9	132.7	0.9	26.5
S. R.	♀	37	−	8.5	5.0	131.7	0.8	25.8
H. E.	♀	37	−	7.5	5.2	150.8	1.2	28.6
♀	43.8	37–55		10.1 ± 2.7	5.5 ± 0.7*	125.4 ± 19.6	1.08 ± 0.22	26.0 ± 1.3
♀	31.9	26–49	Controls (n = 10)		4.6 ± 0.4			25.6 ± 1.4

tions had also the highest concentrations of DPG. Plasma calcium and phosphorus concentrations were normal in all patients (except one low phosphorus concentration).

DPG exerts an effect on the hemoglobin dissociation curve similar to that of CO. Increasing concentrations of DPG shift the P_{50} to the right. It has been postulated, that hypoxia is important in the production of arteriosclerosis (Hueper 1944). Prolonged tissue hypoxia has also been suspected in the development of diabetic microangiopathy (Ashton and Path 1966). Several clinical conditions such as anemia (Torrance *et al.* 1970) and low output cardiac disease (Woodson *et al.* 1970) have been detected where compensatory changes in the 2,3-DPG level of the red blood cell occur.

No significant differences were found in the erythrocyte 2,3-DPG concentration between normal and diabetic patients, but in the diabetic patients, there could be demonstrated significant fluctuations of DPG concentrations as determined by the metabolic control of their diabetes (Standl and Kolb 1973). Diabetics with pronounced type I or type V hyperlipemia showed markedly increased hemoglobin-oxygen affinity (Ditzel and Dyerberg 1977). Theoretically an increase in 2,3-DPG should increase oxygen release in the peripheral tissue at any given partial pressure of oxygen. It still remains open to question whether the ability of women to increase the concentration of DPG in fact protects them against hypoxia in the arterial wall and thus the development of atherosclerosis.

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Innervation of the Duodenal Submucosal Brunner's Glands

By

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Innervation of the gastrointestinal smooth muscle has been studied extensively using moder specific neurohistochemical and electron microscopical techniques (for ref see *e.g.* Kyöso and Rechardt 1974) At the same time, very little is known about the innervation of th duodenal submucosal Brunner's glands. The present study was undertaken in order t partly fill this gap in knowledge.

Small tissue pieces from the submucosal layer of the duodenum of the cat were excise under general anesthesia induced by i.p injection of sodium thiopentone and immediatel processed for I) fluorescence microscopy using two fluorescence histochemical method 1) the formaldehyde-induced fluorescence histochemical method (Eränkö 1967 a), 2) th glyoxylic acid-induced fluorescence histochemical method (Lindvall and Björklund 1974

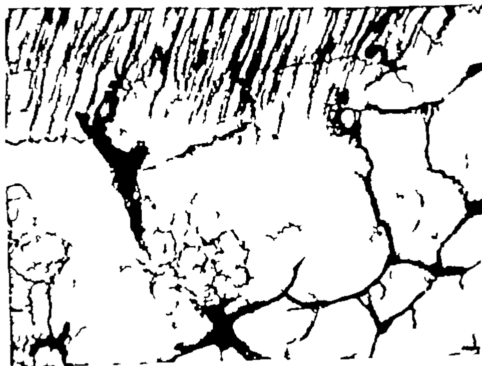


Fig. 1 Delicate small-meshed nerve nets consisting of terminal varicose ramifications of acetylcholin-esterase-positive axons enclose the Brunner's glands in "basket-like" fashion, an arrangement suggestive of functional cholinergic (vagal) innervation.



...ence distribution of fluorescing (adrenergic) axons in the submucosal layer of the duodenum, ...spatial relation to the Brunner glands

...l et al. 1974), and II) for the demonstration of acetylcholinesterase-positive nerves ...t sections using two thiocholine techniques: 1) Gomori's modification (1952) of ...elle-Friedenwald technique (1949), 2) the direct-coloring copper-ferrocyanide ...introduced by Karnovsky and Roots (1964).

...ase small-meshed nerve nets consisting of terminal varicose ramifications of acetyl-...terase-positive axons were seen to enclose the Brunner's glands in basket-like ..., an arrangement suggestive of functional innervation (Fig. 1). In contrast, the distri-...of fluorescing axons in the submucosal layer of the duodenum was relatively scarce, ...was impossible to demonstrate a specific functional adrenergic innervation of the ...r's glands (see Fig. 2).

...ne acetylcholinesterase activity can be considered to be a tentative suggestion of the ...rgic nature of neuron (Eränko 1967 b). Thus, the acetylcholinesterase positive ...closely apposing onto the Brunner's glands obviously represent "truly cholinergic" ...elements, instead of being only cholinoceptive. This concept is further corroborated ...observation that only very few adrenergic axons were seen i... the immediate vicinity ...e glands. The conclusion thus seems justified that the neural regulation of the se-...r activity of the Brunner's glands is (at least mainly) cholinergic, and consequently ...ably of vagal origin.

...menfassung. Die Innervation der duodenalen submucosen Drüsen Brunners der ...wurde mit zwei Fluoreszenzmethoden zur Sichtbarmachung von Monoaminen und ...zwei histochemischen Methoden zum Nachweis von acetylcholinesterase-positiven ...fasern in Gewebeschnitten untersucht. Die cholinerge Innervation der Brunnerschen ...n ist reichlich während keine funktionelle adrenerge Innervation festgestellt werden

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Rapid and Repetitive Measurements of Blood Flow and Oxygen Consumption in the Rat Brain Using Intraarterial Xenon Injection

By

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[illegible] method for rapid and repetitive measurements of cerebral blood flow (CBF) and cerebral metabolic rate for oxygen ($CMRO_2$) in rat in situations of non-steady state, e.g. seizures, has not yet been described. We have therefore applied the intra-arterial xenon injection method (Lassen-Rasmussen et al. 1966) to the rat.

The main problem in animal studies with this technique, which has been extensively used in man, is to avoid the influence of contamination from extracerebral tissues on the xenon clearance curves. Such a contamination will result in too low flow values due to the slower [illegible] of extracerebral tissues. In [illegible] previous study of blood-brain-barrier permeability in the rat we described an animal preparation in which extracerebral contamination is minimal (Hertz and Bolwig 1976) and we therefore proceeded to use it for measurement of CBF and CMRO

[illegible]–400 g Wistar rats were anesthetized with 1 2 [illegible] halothane in [illegible] gas mixture of 70 [illegible] N_2O and 30 % [illegible]. All extracerebral branches from both carotid arteries [illegible] ere ligated as described in detail by Hertz and Bolwig (1976). The [illegible] ght external carotid artery [illegible] as cannulated in [illegible] retrograde fashion [illegible] ith the tip of the [illegible] at the carotid bifurcation allowing the bolus to be injected into the internal carotid artery [illegible] ithout [illegible] of the artery. Cerebral venous blood from the confluence of sinuses was sampled from [illegible] can-[illegible] placed in [illegible] burr hole in the [illegible] process.

Mean arterial blood pressure (MABP) and rectal temperature were monitored throughout the study. A [illegible] in MABP by more than 20 [illegible] of the initial value [illegible] as avoided with blood transfusions. Blood [illegible] through [illegible] catheter in [illegible] femoral artery was used for determination of pH, $PaCO_2$, and PaO_2. The [illegible] as always above 100 mmHg. After the surgical procedures halothane was switched off and the ani-[illegible] was paralysed [illegible] ith succinyl choline 0 1 mg/kg and ventilated on [illegible] respirator. 20 min elapsed before [illegible] measurements.

For determination of CBF [illegible] 50 μl bolus of the γ-emitting isotope [133]Xenon was injected into the internal [illegible] artery and the [illegible] about [illegible] as followed by external detection with [illegible] collimated NaI(Tl) crystal (aper-[illegible] mm) placed on the head between eye and ear on the side ipsilateral to the injection site. By means of [illegible] lead plate detection of activity in tracheostomy tube and carotid catheter was [illegible] voided.

For $CMRO_2$ determination arterial and cerebral venous blood [illegible] as sampled 30 [illegible] after bolus injection [illegible] the samples blood gas tensions and total oxygen content (Borgström et al. 1974) [illegible] ere measured.

CBF was calculated from the initial slope (Olesen et al. 1971) of [illegible] semilog plot of the clearance curve [illegible] approximately the first 15 [illegible] of the curve. $CMRO_2$ [illegible] as calculated by multiplying CBF with arterio-[illegible] difference in oxygen content.

Hypercapnia [illegible] as induced by adding 1–5 [illegible]
[illegible]

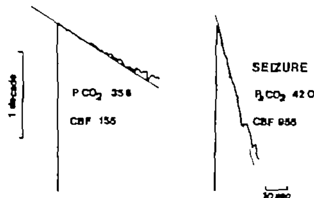


Fig. 1 Semilogarithmically recorded xenon wash-out curves. $PaCO_2$ in mmHg; CBF in ml/100 g min.

Fig. 1 shows representative [133]Xenon clearance curves in normocapnia and during seizure. Fig. 2 shows CBF values as a function of $PaCO_2$ in 43 measurements in 20 rats. $CMRO_2$ in 15 animals with $PaCO_2$ between 22 and 42 mmHg was 10.6 ± 0.7 ml/100 g min (mean $\pm$S.E.)

The CBF and $CMRO_2$ values found are in good agreement with the results of Eklöf *et al* (1973). It is possible to get reproducible results with the method and even very high CBF values can be measured.

Several methods for measurement of CBF in the rat are available. The most extensively studied method is the Kety-Schmidt method used by Eklöf *et al* (1973) and later by Gjedde *et al* (1975). This method is based on determination of arteriovenous differences for xenon during desaturation following inhalation of the inert gas and requires steady state conditions during 10 min.

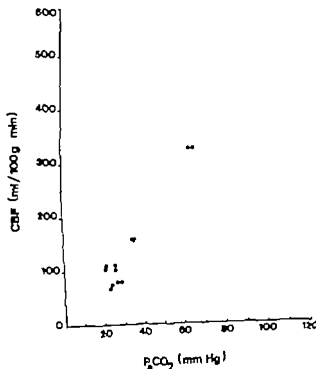


Fig. 2 CBF values obtained at different carbon dioxide tensions in the arterial blood.

e uptake methods are fraught with difficulties. The antipyrine method (Reivich et al sts on the incorrect assumption that antipyrine freely crosses the blood-brain-barrier ecially in high flow situations CBF is underestimated In the xenon uptake study moto et al. 1975) it is difficult to avoid evaporation of tracers from the tissue during on

-arterial injection of inert gas has previously been tried by Chapman et al. 1975 r their results showed persistently low CBF values the authors disregarded the of extracerebral contamination.

e present preparation extracerebral contamination was minimized by extensive of the extracerebral branches from the carotid arteries and this is a prerequisite ing I.a. injection technique in animals. Furthermore, using the inert xenon gas there problems with incomplete diffusion exchange between blood and brain as with anti-alcohol or tritiated water The i.a. injection method offers several advantages It t require steady state for more than 20–30 s during which the outwash curve is ob-It allows repeated measurements in the same animal.

method determines mainly the fast component of CBF which largely corresponds to l grey matter flow Similarly the a. differences in oxygen content obtained using blood from the confluence of sinuses are mainly representative for cortical structures. oth CBF and CMRO values obtained with this method are slightly higher than the brain values (N lsson 1974) which must include more of the slowly perfused sparse atter with lower metabolism.

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Inhibition of Bradykinin Induced Macromolecular Leakage from Post-Capillary Venules by a β_2-Adrenoreceptor Stimulant, Terbutaline

By

E. Svensjö, C. G A. Persson and G Rutili

Histamine and bradykinin both increase microvascular permeability to proteins. Majno and Palade (1961 used carbon particles to study site and size of macromolecular leakage and found by electron microscopy that this increase occurred in venules of 20–30 μm diameter and suggested that this was due to the formation of "gaps" resulting from the contraction of endothelial cells because of stimulation by histamine and bradykinin. We obtained similar results in a study based on intravital microscope observations of the extravasation of intravenously injected fluorescein-labelled macromolecules (Svensjö *et al.* (1973) Furthermore, using the technique of Simionescu *et al* (1971) to demonstrate polysaccharides, we have found by electron microscopy that macromolecular leakage occurs through gaps between endothelial cells following topical application of bradykinin (Hultström and Svensjö 1976) The precise mechanism by which bradykinin and histamine induce contraction of endothelial cells is not known. The presence of contractile elements in these cells similar to those in smooth muscle suggests a contractile mechanism analogous to that of smooth muscle cells (Becker and Nachmann 1973) If this assumption is valid, it might be possible to counteract histamine and bradykinin induced endothelial cell contraction with drugs such as β-adrenoreceptor stimulants which are known to relax smooth muscle cells. Green (1972) showed that leakage of Evans Blue from vessels in the mouse peritoneum was antagonized by catecholamines and salbutamol acting predominantly on β-adrenoreceptors, and suggested that this effect was due to antagonism of endothelial cell contraction We have used terbutaline as a β-adrenoreceptor stimulant in a study of its effect upon bradykinin-induced macromolecular leakage

Golden hamsters weighing 70–100 g were anesthetized with pentobarbital, 9 mg·100 g^{-1} b.w i.p. A femoral vein was cannulated for supplementary injections of pentobarbital and for injection of indomethacin 2 mg·100 g^{-1} b.w Experiments were carried out on the everted hamster cheek pouch preparation as described by Duling (1973) and with our modifications (Svensjö *et al* 1977 a). This experimental model has been found suitable for quantitative intravital studies of the microvascular leakage of macromolecules such as fluorescein labelled dextran (FITC-dextran 150, $\bar{M}_w$ = 150 000, Pharmacia Fine Chemicals, Uppsala, Sweden) induced by histamine and bradykinin (Svensjö *et al* 1973). The preparation also enables simultaneous measurements of red cell velocity in single arterioles as described by Arfors *et al* (1975). Blood flow measurements, estimated from red cell velocity and vessel diameter were relevant to estimate the effect of bradykinin and terbutaline on blood flow In the first series of experiments 5 hamsters were used. Following i.v. injection of FITC-dextran 150 the number of FITC-dextran leakage sites in the exposed area of the

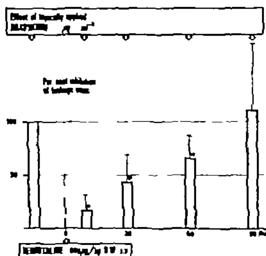


Fig. 1. Repeated topical application of bradykinin to the hamster cheek pouch preparation before and after the intravenous injection of terbutaline 500 μg/kg b.w. Response expressed as percent of preterbutaline response to bradykinin ($\pm$S.D.). p [illegible] 0.05, Student's t-test for paired observations.

cheek pouch was between 0 and 10 sites per cm² (preapplication control value). Bradykinin 0.1 μg ml⁻¹ was applied topically for 4 min during which the normal superfusion of the cheek pouch preparation was interrupted. The number of FITC-dextran leakage sites at postcapillary venules was counted 1 min after the application of bradykinin had been completed. The number of microvascular leakages was maximal at that time. The site of leakage was consistently confined to the postcapillary venule. After the extravasated FITC-dextran had been cleared from the tissue by the superfusing solution, another application of bradykinin, this time with terbutaline (1 μg ml⁻¹) added, could be made.

The results from 5 hamsters showed that the total number of leakage sites at postcapillary venules was 86 ± 31 (S.E.) sites per cm² with bradykinin alone and 29 ± 11 with the combination of bradykinin and terbutaline. (Student's t-test for paired observations, t 2.803, P 0.049). These figures should be compared with the pre-application control values of 0 to 10 sites per cm.

In another series of 5 hamsters the cheek pouches were exposed to bradykinin in the same way but with terbutaline given i.v. in a dose of 50 μg per 100 g b.w. The preparation was then repeatedly exposed to a bradykinin solution (0.1 μg ml⁻¹) (Fig. 1). 10 min after i.v. injection of terbutaline there was a significant reduction in the number of leakage sites to 17 14 of the pre-injection value with bradykinin alone (Fig. 1). Repeated application of the same dose of bradykinin showed that the approximate duration of the effect of this dose of terbutaline was 1 h (Fig. 1).

Microvascular blood flow was estimated in one arteriole in each of 8 other hamsters. Bradykinin, applied as described above, increased arteriolar blood flow to 199% and terbutaline increased it to 217% of control blood flow. Only the bradykinin-induced increase was statistically significant (Table I).

Topical application of bradykinin resulted thus in an increase in both macromolecular leakage and blood flow. Terbutaline was found to counteract the induced increase in macromolecular leakage at postcapillary venules although terbutaline applied separately increased blood flow by 217 (P 0.12). It is unlikely that the combination of bradykinin and terbutaline should result in smaller blood flow increase than caused by bradykinin alone.

TABLE I Arteriolar blood flow in nl s⁻¹ Vessel size 36.6±2.3 μm showing blood flow at start of expt. (control) and after topical application of bradykinin and terbutaline. Student's t test for paired observations.

	Control at start	Control immediately before	Bradykinin 0.1 μg ml⁻¹	Control immediately before	Terbutaline 1 μg ml⁻¹
n	8	8	8	6	6
x	2.47	2.04	4.04	1.02	2.21
S.D	1.31	1.56	2.00	0.39	1.76
t	—	—	3.311	—	1.862
P	—	—	0.013	—	0.12
Blood flow in per cent of control	100		199		17

These findings suggest that the inhibition of bradykinin-induced macromolecular leakage by terbutaline would not be explained by an inhibition of the bradykinin-induced increase in blood flow but rather by a specific inhibition of the endothelial cell response of the postcapillary venules to bradykinin. After the completion of the present study further anti-leakage effects have been examined. The inhibitory effect of terbutaline on macromolecular leakage at postcapillary venules was also found with PGE induced leakage although the peak blood flow following PGE -application was the same with as well as without terbutaline (Svensjö *et al* 1977b) Terbutaline has been found to counteract histamine induced leakage of Evans Blue in guinea pig skin (O Donnell and Persson 1977). Mediator induced leakage also seems to occur at gaps between venular endothelial cells in the lung (Pietra *et al* 1971) If a direct inhibitory effect on leakage is exerted by terbutaline in the lung as well as in the cheek pouch, it might reduce mediator-induced oedema and contribute to the effectiveness of terbutaline in asthma.

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Interactions of Potassium, Noradrenaline and Acetylcholine in the Guinea Pig Vas Deferens

By

P. Hedqvist and U. S. v. Euler

twitch response of the isolated guinea pig vas deferens to brief trains of stimuli delivered [illegible] stimulation is, as a rule, inhibited by low and enhanced by higher concentrations [illegible] noradrenaline (NA). The enhancement of the twitch response by NA is due to a post[illegible] stimulant effect, which is annulled by α-adrenoceptor blockers, whereas the [illegible] in twitch height is due to NA acting on postjunctional β_2-adrenoceptors as well as α- and β-adrenoceptors, which are presumably prejunctionally located (Ambache and [illegible] 1971 Hedqvist and Euler 1976). Since the twitch response itself is not inhibited by either [illegible] β-adrenoceptor blockers it has been proposed that the twitch contraction (in contrast [illegible] the slow contraction at prolonged nerve stimulation) is not mediated by but rather [illegible] by NA (Ambache and Zar 1971 Hedqvist and Euler 1976, Jenkins *et al.* 1975). The guinea pig vas deferens responds with twitch-like contractions to moderate con[illegible]trations of KCl, and it has been suggested that K ions may be involved in the twitch [illegible] to nerve stimulation (Euler and Hedqvist 1975 Stjärne 1976).

We report here some preliminary results of studies concerning interactions between [illegible], NA and acetylcholine (ACh) on the guinea pig vas deferens, supporting the [illegible] role of K ions and possibly ACh in the twitch response.

[illegible] pig vasa deferentia were mounted in 5 ml organ bath using Tyrode as bath fluid (for composition, Hedqvist and Euler 1976). The solution was kept at 37°C and gassed with 5 CO_2 in O_2. Transmural [illegible] were delivered by Grass S4 stimulator through platinum electrodes along the wall of the bath. [illegible] contractions were elicited at 1 min intervals by 10–20 biphasic pulses (5–10 Hz, pulse duration 1 ms, supramaximal voltage), which specifically activate the nerves. In some cases the smooth muscle cells were stimulated directly by prolonging the pulse duration to 30 ms, after blockade of nerve conduction with tetrodotoxin (TTX). The contractions were recorded with an isotonic transducer (Harvard heart/smooth [illegible] transducer) on Honeywell ink writer. The load was 0.25 g.

[illegible]dition of KCl to the bath fluid significantly and dose-dependently increased the twitch [illegible]ponse to transmural nerve stimulation. In the higher dose range this enhancement was [illegible]ociated with a direct contraction of the vas deferens (Fig. 1). I[n] the non-stimulated prepa[illegible]tion KCl caused well reproducible, twitch-like contractions when the whole bath fluid [was] rapidly replaced from a special container and the organ exposed to concentrations of [K]Cl 3–5 times the normal (Fig. 2). A twitch response at these concentrations points to a [hig]h sensitivity of the reacting system to K ions. The KCl-induced twitch was annulled by [ad]dition of 0.04–0.06 μg/ml TTX, as were twitches elicited by nerve stimulation (*cf.* Hed[qvi]st and Euler 1976).

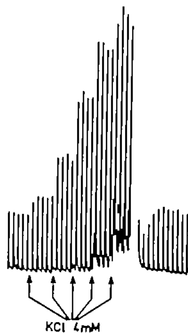


Fig. 1

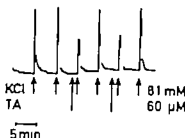


Fig. 2



Fig. 3

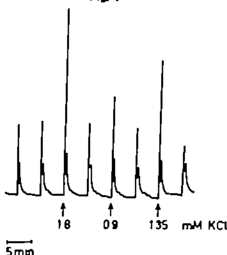


Fig. 4

Fig. 1 Effect of cumulative doses of KCl (4 mM) on twitch response to transmural nerve stimulation, 5 Hz, 1 ms, 10 pulses, at 1 min intervals in guinea pig vas deferens. Wash at dot.

Fig. 2. Effect of tyramine (TA) (60 μM) on twitch-like contraction induced by substituting normal Tyrode by Tyrode containing 8 1 mM KCl.

Fig. 3 Inhibition of NA-induced twitch-like contraction by raising KCl concentration in bath fluid from 2.7 to 3.8 mM. Wash at dots.

Fig. 4 Twitch-like response to ACh (1 1 μM) enhanced by simultaneous addition of KCl (0 9 1.8 mM) at arrows. Wash after each application.

It seemed of interest to study whether the KCl-induced twitch was modified by NA or NA-releasing amines in the same way as the twitch induced by nerve stimulation (*cf* Hedqvist and Euler 1976) This proved to be the case Thus, tyramine (60 μM) markedly reduced the twitch induced by Tyrode solution containing 8 1–10 8 mM KCl (Fig. 2) and a similar inhibition of the KCl twitch was obtained with NA (6–12 μM)

It is well established that NA can produce twitch like contractions, although the dose needed is usually high of the order of 60 μM or more The twitch elicited by NA was annulled or markedly reduced by addition of 1–3 mM KCl (Fig. 3)

Compared to NA ACh is much more prone to induce twitch contraction, and it consistently enhances the twitch induced by nerve stimulation (*cf* Sjöstrand and Swedin 1968). The twitch elicited by 1 1 μM ACh was, like that of KCl, inhibited by NA 6–12 μM It was also observed that the ACh-induced twitch is highly sensitive to potassium. Thus, less than

mM KCl added simultaneously with 1.1 μM ACh strongly enhanced the response (Fig. 4). The twitch response elicited by ACh was still obtainable after TTX, suggesting a direct response of the muscle cells (*cf.* Goodman and Weiss 1971).

The observation that TTX annulled the twitch induced by KCl indicates that KCl in the concentrations used (8.1–10.8 mM) is unable to activate the muscle cell directly. In contrast to ACh or electrical stimulation at increased pulse duration (*cf.* Goodman and Weiss 1971). It may thus be assumed that KCl in these concentrations normally elicits the twitch by depolarizing either the nerve terminal, allowing transmission, or the specialized postjunctional area of the muscle membrane, or both. The inability of KCl to evoke a twitch after TTX may therefore be due either to a prejunctional or a postjunctional membrane block, which might reduce, but not prevent, the action of ACh or electrical stimulation with pulses of extended duration, assuming that the latter effects are mainly extrajunctional. The annulment by TTX of the twitch response to brief electrical pulses and to low concentrations of KCl may therefore in both cases be due to a blockade of the action of potassium, on the assumption that potassium is a prerequisite for triggering the nerve-induced twitch.

It has been shown that relatively high concentrations of KCl, of the order of 20 mM strongly increases the ability of NA to produce a twitch (Stjärne 1976). The present observations that approximately 5 times lower concentrations of KCl are sufficient to strongly potentiate the effect of ACh, that even lower KCl concentrations suffice to annul the NA induced twitch, and that also NA may inhibit the KCl-induced twitch point to an intricate relationship between NA, potassium and ACh and to a pivotal role of K^+ ions in the twitch transmission. The strong enhancement of the twitch response to ACh by low concentrations of KCl is of interest also in view of the postulated participation of ACh in the twitch response to nerve stimulation (*cf.* Birmingham 1966). An interaction of ACh with K^+ ions during the nerve stimulation appears a possibility.

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frequently found close to blood vessels (Olgart *et al* 1977) Antidromic stimulation of t inferior alveolar nerve regularly increases blood flow in the dental pulp, an effect that not influenced by atropine or blockade with β-receptor antagonists (Gazelius and Olga to be published) The increase of SLPI in the pulp superfusates during such stimulation n be taken as an evidence that SP is released and takes part in the vascular response. SP m following the suggested release and after having exerted its effect diffuse into the int stitial fluid rather than being directly eliminated into the blood stream. This may contrib to the explanation why in a previous study no elevation of SPLI levels were noted in blo samples collected following antidromic nerve stimulation of nerves to the tongue, skin a nose of dogs and cats (Burcher *et al* 1977)

In summary the present study supports previous claims for the existence of SPLI in t dental pulp and demonstrates the release of SPLI from this tissue. This observation m support the hypothesis presented by Lembeck (1977) that SP is involved in the axon ref mechanism that was originally described by Dale (1935) If so SP may play a role for t developement of the inflammatory response associated with injury and pain in periphe tissues.

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Supplement appended.

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Tables should be kept at minimum, both in number and size, with text above the table (not on s sheets). Single numbers in a series should be replaced by mean and S.D or mean and S.E., in the latt with number of observations.

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The international system of units (SI)

The following symbols and units, recommended by the SI are being used in Acta Physiologica Scand Certain units, not included in SI, will still be permitted.

SI units with recommended symbols

Units	*Symbols*
kilogramme	kg
second, millisecond	s ms
mole, millimole, micro-mole, nanomole, picomole	mol mmol μmol nmol pmol
meter millimeter micrometer nanometer	m mm μm nm
candela	cd
steradian	sr
hertz (frequency)	Hz (s^{-1})
newton (force)	N (kg m/s^2)
pascal (pressure)	Pa (N/m^2)
joule (energy)	J (N m)
watt (effect)	W (J/s)
lumen (lightflow)	lm (cd sr)
lux (illumination)	lx (lm/m^2)

Conversion factors to be given in Methods.

Permitted non-SI units

Units	*Symbols*
gramme	g
minute	min
hour	h
molarity (mol/liter)	M
(caloric)	cal (4.184 J)
(kilopond)	kp (9.81 N)
(millimeters of mercury)	mm Hg (1.333 bar)
(millibar)	mbar (100 Pa)
curie	Ci
liter milliliter micro-liter	l ml μl
degree Celsius	°C